Glutamic Acid (Glu) Content Assay Kit (WST-1 chromogenic method)

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

Detection equipment: Spectrophotometer

Cat No: NA0168

Size: 100T/48S

Components:

Reagent I: Liquid 85 mL×1, store at 2-8℃.

Reagent II: Liquid 1.5 mL×1, store at 2-8℃.

Reagent III: Powder×2, store at -20℃; Before use, take 1 bottle and add 6mL of reagent I to fully dissolve it. Unused reagents can be stored in aliquots at -20°C for four weeks, avoiding repeated freezing and thawing.

Reagent IV: Powder×2, store at -20℃; Before use, take 1 bottle and add 0.5mL of reagent II to fully dissolve it. Unused reagents can be stored in aliquots at -20°C for two weeks, avoiding repeated freezing and thawing.

Reagent V: Liquid 4mL×1, store at 2-8℃.

Standard: Liquid 0.5 mL×1, store at 2-8℃. 10 μmol/mL glutamic acid standard.

Description:

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 various amino acids through transamination, and is one of the main sources of amino acids in living organisms. In addition, Glu is also the main active ingredient of monosodium glutamate, and is commonly used in food additives and spice production.

Glutamate dehydrogenase (GDH) catalyzes glutamate and NAD to generate α-ketoglutarate, NADH and NH_4^+. Under the action of 1-mPMS, WST-1 can react with NADH to produce water-soluble formazan, and calculate glutamate acid content.

Required but not provided:

Spectrophotometer / microplate reader, centrifuge, transferpettor, water bath / constant temperature incubator, micro glass cuvettes/96 well plates, mortar/homogenizer, sonicator, ice and distilled water.

Protocol:

I. Sample preparation

1、 Bacteria and cells: Collect bacteria or cells into a centrifuge tube, discard the supernatant after centrifugation; add 1 mL of reagent I for every 5 million bacteria or cells, and ultrasonically disrupt the bacteria or cells (power 200w, ultrasonic 3s, interval 10s, repeat 30 times), 10000g, centrifuge at room

temperature for 10min, and take the supernatant for testing.

2、 Tissue: Weigh about 0.1 g of tissue, add 1 mL of reagent I, homogenize in an ice bath, 10000 g, centrifuge at room temperature for 10 min, and take the supernatant for testing.

3、 Liquid: Direct determination. (If the solution is cloudy, take the supernatant after centrifugation for determination)

II. Detection

1. Preheat spectrophotometer/microplate reader for 30 min, adjust wavelength to 450 nm, spectrophotometer set zero with distilled water.

2. Standard solution: dilute as 0.625, 0.3125, 0.156, 0.078, 0.039, 0.0195, 0.01, 0.005, 0.0025 μmol/mL standard solution with distilled water.

3. Test operation table ：

Reagent（μL）	Standard tube(S)	Standard blank tube(ST)	Test tube(T)	Control tube(C)
Standard	40	-	-	-
Distilled water	-	40	-	-
Sample	-	-	40	40
Reagent I	-	170	-	170
Reagent III	160	-	160	-
Reagent IV	10	-	10	-
Reagent V	30	30	30	30
Mix well and react at 37°C for 30min (Light avoidance)				

Take 200 μL into a glass cuvette/96 well plate, read the absorbance value At、 Ac at 450 nm, calculate ΔA=At-Ac. ΔAs = As - Ast. (Standard tube and standard blank tube only need to do 1-2 times).

III. Calculation

1. Standard curve.

According to the concentration of the standard tube (x, μmol/mL) and the absorbance ΔAs (y, ΔAs), establish a standard curve. From the standard curve, plug ΔA into the equation to get x (μmol/mL).

2. Calculation of glutamic acid content

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)=$x \times VS \div (W \div VST \times VS) = x \div W$

C. Bacteria or cells amount

Glu (μmol/10^4 cell)＝$x \times Vs \div (500 \div VST \times VS) = 0.002x$

D. Liquid volume:

Glutamate content (μmol/mL) = $x \times Vs \div Vs = x$

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

V_S: Sample volume, 0.04 mL；

Cpr: Sample protein concentration, mg/mL；

W: Sample weight, g；

500: Bacteria or cells amount, 5 million.

Note:

1. If the measured absorbance value exceeds the linear range absorbance value, you can increase the sample volume or dilute the sample before measuring.

Technical Specifications：

Minimum Detection Limit：0.0025 μmol/mL

Linear Range：0.0025-0.625 μmol/mL

Experimental example:

1. Weigh about 0.1g of mouse lung, add 1mL of Reagent I, grind in ice bath, 10000g, centrifuge at room temperature for 10min to get the supernatant for testing. Then follow the measurement steps, measure with a glass cuvette and calculate ΔA. ΔA=At-Ac=0.449-0.384=0.065, standard curve y=1.5338x+0.0091, X=0.036 according to the standard curve, glutamic acid Content is:
   Glutamate content (μmol/g mass) = x×Vs÷ (W÷Vst×Vs)=x÷W=0.36μmol/g.
2. Aspirate 0.04 mL of goat serum, operate according to the determination steps, measure with a glass cuvette and calculate ΔA, and calculate ΔA= At-Ac=0.379-0.105=0.274, standard curve y=1.5338x+0.0091, according to the standard curve X=0.173, the glutamic acid content is:
   Glutamate content (μmol/mL) = x × Vs ÷ Vs = x = 0.173 μmol/mL.

Recent Product Citations：

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：

NA0845/NA0603 Proline(PRO) Content Assay Kit

NA0856/NA0614 Cysteine(Cys) Content Assay Kit