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Glutamic Acid (Glu) Assay Kit (WST-8 Method)

Catalog No.: BC00029

Size: 50T

If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉Email (Sale) order@enkilife.com

✉Email (Techsupport) techsupport@enkilife.com

Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Glutamic Acid (Glu) Assay Kit (WST-8 Method)
Detection Methods	Colorimetric
Sample type	Serum, plasma, urine , animal and plant tissues, cells
Detection Type	Quantitative
Detection instrument and wavelength	Microplate reader (450-490 nm, optimal detection wavelength 470 nm)

Product Introduction

Glutamate is present in large quantities in cereal proteins and in high concentrations in animal brains. Glutamate plays an important role in protein metabolism in living organisms and is involved in many important chemical reactions in animals, plants and microorganisms. It is also the most abundant excitatory neurotransmitter in the nervous system of vertebrates. Glutamate is an important non-essential amino acid, which means that the human body can synthesize enough nutrients for its use.

Features

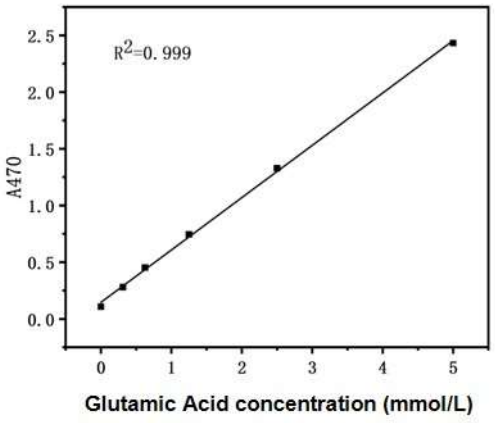
★ WST-8 is an upgraded substitute for MTT, and has obvious advantages over MTT or other MTT-like products such as XTT, MTS, etc. First, the formazan generated by the reduction of MTT by some dehydrogenases is not water-soluble and requires a specific dissolving solution to dissolve; while the formazan generated by WST-8, XTT, and MTS are all water-soluble, which can save the subsequent dissolution step. Secondly, the formazan generated by WST-8 is more soluble than the formazan generated by XTT and MTS. Thirdly, WST-8 is more stable than XTT and MTS, making the experimental results more stable.

★ Compared with MTT, XTT, etc., WST-8 has a wider linear range and higher sensitivity.

★ Compared with WST-1, WST-8 has higher detection sensitivity, is more soluble, and is more stable.

Detection Principle

Under the catalytic action of enzymes, glutamic acid (Glu) can reduce NAD+ to NADH. NADH, under the action of hydrogen-donating substances, reduces WST-8 to form an orange-yellow substance, which has the maximum absorption peak at 470 nm. Within a certain range, the absorbance value is directly proportional to the concentration. By measuring the absorbance at 470 nm or nearby wavelengths, the content of Glu in the sample can be calculated.



Product Composition

Serial Number	Product Name	Packing specification (50T)	Storage
Reagent 1	Buffer	8 mL	-20℃, keep away from light
Reagent 2	Enzyme reagents	powder	-20℃, keep away from light
Reagent 3	Substrate	powder	-20℃, keep away from light
Reagent 4	Color developer	1.2 mL	-20℃, keep away from light
Reagent 5	50 mmol/L standard	1 mL	-20℃, keep away from light
Consumables 1	96-well ELISA plate	1 plate	RT
Consumables 2	96-well membrane	2 pieces	RT

Storage conditions

The unopened kit can be stored at -20°C for 6 months.

Preparation before the experiment

Sample processing

1. Sample processing Tissue samples: Take 0.1 g of fresh tissue sample, add 0.9 mL of physiological saline (0.9% NaCl) to homogenize, centrifuge at 12000×g for 15 min at 4°C, take the supernatant and filter it through a 10 KD ultrafiltration tube, collect the filtrate for testing .
2. Cell samples: Collect 1×10 ^{6} cell samples and add 200 μL physiological saline (0.9% NaCl) to homogenize, centrifuge at 12000 × g for 15 min at 4°C, take the supernatant and filter it through a 10 KD ultrafiltration tube, and collect the filtrate for testing .
3. Serum (plasma), urine and other liquid samples: Use 10 KD ultrafiltration tube for centrifugal

filtration and collect the filtrate for testing.

4. Before the formal test, it is necessary to select 2-3 samples with large expected differences and dilute them into different concentrations for preliminary experiments. According to the results of the preliminary experiments and the detection range of this kit (0.1-5mmol /L) , appropriate dilutions are performed. The sample diluent is physiological saline (0.9% NaCl). In general, human serum can be diluted 3-5 times , and human urine is not diluted.

· Preparation of the kit

1. Before testing, all reagents were equilibrated to room temperature. The 50 mmol/L standard needed to be heated in a 60°C water bath for about 10 min until completely dissolved before use.
2. enzyme reagent working solution: Take one enzyme reagent and add 1.2 mL of double distilled water to fully dissolve it. Place it on an ice box for use. The unused portion can be stored at -20°C away from light for 7 days.
3. substrate working solution: Take one tube of substrate and add 1.2 mL of double distilled water to fully dissolve it. Place it on an ice box for use. The unused portion can be stored at -20°C away from light for 7 days.
4. Preparation of reaction working solution: Prepare enzyme reagent working solution: substrate working solution: color developer in a volume ratio of 1:1:1. Prepare it before use and prepare it as needed. Place the prepared working solution on an ice box away from light and use it within 3 hours.
5. Preparation of 5 mmol/L standard solution: Prepare according to the volume ratio of 50 mmol/L standard solution: double distilled water = 1:9. The prepared standard solution is valid for the same day.
6. Preparation of standards of different concentrations: Dilute the 5 mmol/L standard into the following concentrations: 5 , 2.5 , 1.25 , 0.625 , 0.3125 , 0. (Blank well) mmol/L . The prepared standard solution is valid for the day .

Operation process

1. Standard wells: add 30 μL of standards of different concentrations to the standard wells; assay wells: add 30 μL of the sample to be tested to the assay wells.

2. Add 130 μL of buffer to each of the standard wells and assay wells in step “ 1 ” .

3. to each well in step " 2 " .

4. Vibrate the plate for 3 s, incubate at 37°C in the dark for 20 min (the time can be adjusted appropriately) , and measure the absorbance of each well at 470 nm or a similar wavelength (450nm-490nm is acceptable, 470nm is optimal) using a microplate reader .

	Standard well	Determination well
Standard (μL)	30	--
Sample to be tested (μL)	--	30
Buffer (μL)	130	130
Reaction working solution	60	60
Vibrate the plate for 3 s, incubate at 37°C in the dark for 20 min (the time can be adjusted appropriately), and measure the absorbance of each well at 470 nm or a similar wavelength (450nm-490nm is acceptable, 470nm is optimal) using a microplate reader.		

Result calculation

Standard fitting curve: y = ax + b

Glu content in tissue sample (mmol/kg wet weight) = (ΔA4 7 0 - b) ÷ a ÷ m/V × f

Glu content in cell sample (mmol/10^6) = (ΔA4 7 0 - b) ÷ a ÷ n / V × f

The formula for calculating the Glu content in serum and plasma samples is: Glu content (mmol/L) = (ΔA4 7 0 - b) ÷ a × f

y: absorbance of standard well - absorbance of blank well (absorbance when the concentration of standard is 0)

x: concentration of the standard

a: slope of the curve

b: intercept of the curve

ΔA4 7 0: measured absorbance - blank absorbance

f: dilution factor of the sample before adding it to the detection system

m: wet weight of tissue, g

n: number of cell samples, 10^6

V: volume of sample homogenate, mL

Notes

1. Please read the instructions carefully and adjust the instrument before the experiment, and conduct the experiment strictly in accordance with the instructions.
2. The detection range of the kit is not equivalent to the concentration range of the analyte in the sample. If the concentration of the analyte in the sample is too high or too low, please dilute or concentrate the sample appropriately.
3. This product is limited to scientific research by professionals and must not be used for clinical

diagnosis or treatment, used as food or medicine, or stored in ordinary residences.