PRODUCT INFORMATION & MANUAL

Glutamate Dehydrogenase Activity Assay Kit (Colorimetric)

NBP3-24494

For research use only.
Not for diagnostic or therapeutic procedures.

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Glutamate Dehydrogenase Activity Assay Kit (Colorimetric)

Catalog No: NBP3-24494

Method: Colorimetric method

Specification: 96T (Can detect 40 samples without duplication)

Instrument: Microplate reader

Sensitivity: 0.54 U /L

Detection range: 0.54-25.0 U/L

Average intra-assay CV (%): 3

Average inter-assay CV (%): 4

Average recovery rate (%): 103

▲ This kit is for research use only.

▲ Instructions should be followed strictly, changes of operation may result in unreliable results.

▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

This kit can measure glutamate dehydrogenase(GDH) activity in serum (plasma), urine, and animal tissuel samples.

▲Detection principle

Glutamate dehydrogenase (GDH) can catalyze the reversible oxidative deamination of glutamate to α-ketoglutaric acid, which is a key enzyme in carbon and nitrogen metabolism. The activity of GDH in serum can be used as one of the important indicators of hepatocyte canceration, and also provide certain application value for the diagnosis of other diseases.

GDH catalyzes the dehydrogenation of glutamate, meanwhile, NAD^+ is reduced to NADH, which under the action of hydrogen transmitter, transfer electrons to WST-8 to produce the yellow product. The activity of GDH can be calculated by measuring the change of absorbance value at 450 nm.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Extracting Solution	50 mL × 1 vial	2-8℃ , 12 months
Reagent 2	Buffer Solution	15 mL × 1 vial	2-8℃ , 12 months
Reagent 3	Substrate A	Powder ×1 vial	2-8℃ , 12 months, shading light
Reagent 4	Substrate B	5 mL ×1 vial	2-8℃ , 12 months
Reagent 5	Chromogenic Agent	1.2 mL × 2 vials	2-8℃ , 12 months, shading light
Reagent 6	Standard	Powder × 2 vials	2-8℃ , 12 months, shading light
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.

▲Materials prepared by users

Instruments

Incubator(37℃), Centrifuge, Microplate reader (440-460nm, optimum wavelength: 450 nm)

Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

The key points of the assay

1. The prepared reagent 3 working solution and 1 mmol/L standard solution can be stored at 2-8℃ with shading light for 7 days.
2. Prepare the fresh needed amount of reagent 5 working solution before use and the prepared solution can be stored with shading light and used within 1 day.

Pre-assay preparation

▲ Reagent preparation

1. Bring all reagents to room temperature before use.
2. Preparation of reagent 3 working solution:
   Dissolve a vial of reagent 3 with 1.5 mL double distilled water and preserve it on ice for detection. The prepared solution can be stored at 2-8°C with shading light for 7 days.
3. Preparation of reagent 4 working solution:
   Mix the reagent 3 working solution and reagent 4 at the ratio of 1:2 fully. Prepare the fresh needed amount before use and the prepared solution can be used within 12 h.
4. Preparation of reagent 5 working solution:
   Mix the reagent 2 and reagent 5 at the ratio of 6:1 fully. Prepare the fresh needed amount before use and the prepared solution can be stored with shading light and used within 1 day.
5. Preparation of 1 mmol/L standard solution:
   Dissolve a vial of reagent 6 powder with 0.5 mL double distilled and preserve it on ice for detection. The prepared solution can be stored at 2-8°C with shading light for 7 days.

▲ Sample preparation

1. Serum and plasma samples:

Detect the sample directly. If the sample is turbidity, centrifuge at 10000 g for 10 min, then take the supernatant for detection.

2. Tissue sample:

Accurately weigh the tissue, add reagent 1 at a ratio of Weight (g): Volume (mL) =1:9 and homogenize the sample in ice water bath. Then centrifuge at 10000 g for 15 min, then take the supernatant for detection. Meanwhile, determine the protein concentration of supernatant. If the supernatant is turbidity after centrifugation, repeat centrifugation until clarified.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.54-25.0 U/L).

The recommended dilution factor for different samples is as follows (for reference only)

Sample type	Dilution factor
10% Rat liver tissue homogenate	3-5
10% Rat kidney tissue homogenate	1
10% Rat heart tissue homogenate	1
10% Rat brain tissue homogenate	1
10% Rat spleen tissue homogenate	1
10% Rat lung tissue homogenate	1
10% Mouse liver tissue homogenate	1
10% Mouse kidney tissue homogenate	1
10% Mouse spleen tissue homogenate	1
10% Mouse lung tissue homogenate	1
Human serum	1
Human plasma	1
Rat serum	1
Rat plasma	1

Note: The diluent is reagent 1.

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S1'	S9	S9'	S17	S17'	S25	S25'	S33	S33'
B	B	B	S2	S2'	S10	S10'	S18	S18'	S26	S26'	S34	S34'
C	C	C	S3	S3'	S11	S11'	S19	S19'	S27	S27'	S35	S35'
D	D	D	S4	S4'	S12	S12'	S20	S20'	S28	S28'	S36	S36'
E	E	E	S5	S5'	S13	S13'	S21	S21'	S29	S29'	S37	S37'
F	F	F	S6	S6'	S14	S14'	S22	S22'	S30	S30'	S38	S38'
G	G	G	S7	S7'	S15	S15'	S23	S23'	S31	S31'	S39	S39'
H	H	H	S8	S8'	S16	S16'	S24	S24'	S32	S32'	S40	S40'

Note: A-H, standard wells; S1-S40, control wells; S1'-S40' sample wells.

▲Detailed operation steps

1. The preparation of standard curve

Dilute 1 mmol/L standard solution with reagent 1 to a serial concentration. The recommended dilution gradient is as follows: 0.00.00, 0.05, 0.10, 0.20, 0.25, 0.30, 0.40, 0.50 mmol/L. Reference is as follows:

Number	Standard concentrations (mmol/L)	1 mmol/L standard solution (μL)	Reagent 1 (μL)
A	0.00	0	200
B	0.05	10	190
C	0.10	20	180
D	0.20	40	160
E	0.25	50	150
F	0.30	60	140
G	0.40	80	120
H	0.50	100	100

2. The measurement of samples

(1) Standard well: Add 20 μL of standard solution with different concentrations to the corresponding wells.

Control well: Add 20 μL of sample to the corresponding wells.

Sample well: Add 20 μL of sample to the corresponding wells.

(2) Add 60 μL of double distilled water to control well.

Add 60 μL of reagent 4 working solution to standard well and sample well.

(3) Add 140 μL of reagent 5 working solution to each well.

(4) Mix fully with microplate reader for 5 s and incubate at 37°C with shading light for 20 min. Measure the OD value of each well at 450 nm with microplate reader.

▲Summary operation table

	Standard well	Control well	Sample well
Standard solution with different concentrations (μL)	20		
Sample (μL)		20	20
Double distilled water (μL)		60	
Reagent 4 working solution (μL)	60		60
Reagent 5 working solution (μL)	140	140	140
Mix fully and incubate at 37°C with shading light for 20 min. Measure the OD value of each well.			

Calculation

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: $y = ax + b$.

1. For tissue:

Definition: The amount of GDH in 1 g tissue protein per 1 minute that hydrolyze the substrate to produce 1 μmol product at 37°C is defined as 1 unit.

$$\text{GDH activity (U/gprot)} = (\Delta A - b) \div a \div T \div C_{pr} \times f \times 1000^{*}$$

2. For serum/plasma:

Definition: The amount of GDH in 1 L liquid sample per 1 minute that hydrolyze the substrate to produce 1 μmol product at 37°C is defined as 1 unit.

$$\text{GDH activity (U/L)} = (\Delta A - b) \div a \div T \times f \times 1000^{*}$$

Note:

y: $OD_{Standard} - OD_{Blank}$ (OD_{Blank} is the OD value when the standard concentration is 0).

x: The concentration of standard.

a: The slope of standard curve.

b: The intercept of standard curve.

ΔA: $OD_{Sample} - OD_{Control}$.

T: The time of incubation reaction, 20 min.

C_{pr}: Concentration of protein in sample, gprot/L.

f: Dilution factor of sample before tested.

1000*: 1 mmol/L = 1000 μmol/L

Appendix I Data

▲Example analysis

For rat kidney tissue, carry the assay according to the operation table.

The results are as follows:

standard curve: y = 1.6746 x - 0.0157, the average OD value of the control well is 0.299, the average OD value of the sample is 0.554, the concentration of protein in sample is 13.76 gprot/L, and the calculation result is:

GDH activity (U/gprot) = (0.554 - 0.299 + 0.0157) ÷ 1.6746 ÷ 13.76 ÷ 20 × 1000

= 0.59 U/gprot