（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K759-M

Specification：96T(40 samples)

Measuring instrument：Microplate reader（440-460 nm）

Detection range：0.54-25.0 U/L

Elabscience®Glutamate Dehydrogenase (GDH) Activity Assay Kit

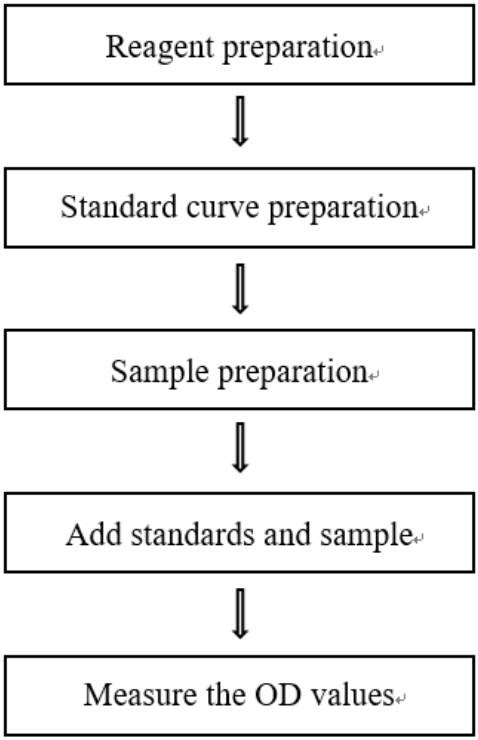
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

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

Table of contents

Assay summary



Intended use

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

Detection principle

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	Size (96 T)	Storage
Reagent 1	Extracting Solution	50 mL ×1 vial	2-8ºC, 12 months
Reagent 2	Buffer Solution	15 mL ×1 vial	2-8ºC, 12 months
Reagent 3	Substrate A	Powder ×1 vial	2-8ºC, 12 months, shading light
Reagent 4	Substrate B	5 mL ×1 vial	2-8ºC, 12 months
Reagent 5	Chromogenic Agent	1.2 mL ×2 vials	2-8ºC, 12 months, shading light
Reagent 6	Standard	Powder ×2 vials	2-8ºC, 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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

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

Reagents:

Double distilled water, PBS (0.01 M, pH 7.4)

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② Preparation of substrate A working solution:

Dissolve one vial of substrate A with 1.5 mL of double distilled water, mix well to dissolve. Store at 2-8℃ for 7 days protected from light

③ Preparation of substrate B working solution:

For each well, prepare 60 μL of substrate B working solution (mix well 20 μL of substrate A working solution and 40 μL of substrate B). The substrate B working solution should be prepared on spot and used up in 12 h.

④ Preparation of chromogenic working solution:

For each well, prepare 140 μL of chromogenic working solution (mix well 120 μL of buffer solution and 20 μL of chromogenic agent). The chromogenic working solution should be prepared on spot and used up in 12 h.

⑤ Preparation of 1 mmol/L standard solution:

Dissolve one vial of standard with 0.5 mL of double distilled water, mix well to dissolve. Store at 2-8℃ for 7 days protected from light.

⑥ The preparation of standard curve:

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 1 mmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.05, 0.10,

0.20, 0.25, 0.30, 0.40, 0.50 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.05**	**0.10**	**0.20**	**0.25**	**0.30**	**0.40**	**0.50**
1 mmol/L standard (μL)	0	10	20	40	50	60	80	100
Double distilled water (μL)	200	190	180	160	150	140	120	100

Sample preparation

① Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80℃ for a month.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL extracting solution with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

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 extracting solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① The prepared substrate A 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 chromogenic working solution before use and the prepared solution can be stored with shading light and used within 1 day.

Operating steps

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 substrate B working solution to standard well and sample well.

③ Add 140 μL of chromogenic working solution to each well.

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

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. Serum (plasma) sample:

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

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

2. Tissue sample:

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.

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

[Note]

ΔA: $OD_{Sample} - OD_{Control}$.

T: The time of incubation reaction, 20 min.

Cpr: The concentration of protein in sample, gprot/L.

f: Dilution factor of sample before test.

1000*: 1 mmol/L = 1000 μmol/L.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	1.50	9.80	13.50
%CV	3.5	3.2	2.3

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	1.50	9.80	13.50
%CV	3.7	4.1	4.2

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 98%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	0.07	0.23	0.35
Observed Conc. (mmol/L)	0.1	0.2	0.4
Recovery rate (%)	101	104	104

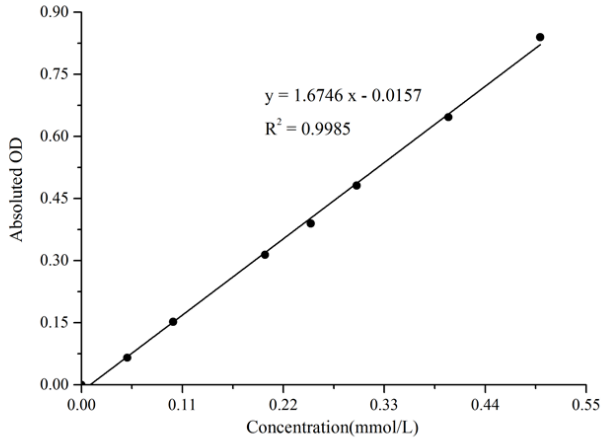
Sensitivity

The analytical sensitivity of the assay is 0.54 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

Concentration (mmol/L)	**0**	**0.05**	**0.10**	**0.20**	**0.25**	**0.30**	**0.40**	**0.50**
Average OD	0.049	0.115	0.201	0.363	0.439	0.531	0.696	0.889
Absoluted OD	0.000	0.066	0.152	0.314	0.390	0.482	0.647	0.840



Appendix II Example Analysis

Example analysis:

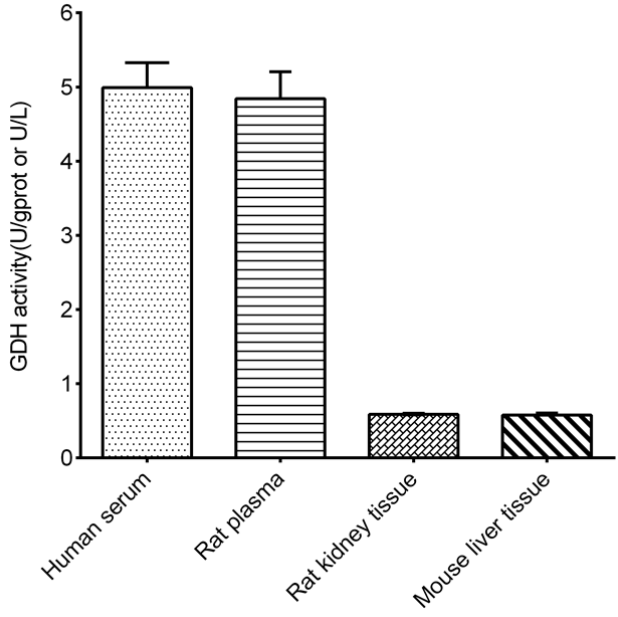
For rat kidney tissue, and carry the assay according to the operation table. The results are as follows:

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:

$$\text{GDH activity (U/gprot)} = (0.554 - 0.299 + 0.0157) \div 1.6746 \div 13.76 \div 20 \times 1000$$

$$= 0.59 \text{ U/gprot}$$

Detect human serum, rat plasma, 10% rat kidney tissue homogenate (the concentration of protein is 13.76 gprot/L) and 10% mouse liver tissue homogenate (the concentration of protein is 20.28 gprot/L) according to the protocol, the result is as follows:



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.