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

Catalog No：E-BC-K278-S

Specification：50 Assays(48 samples)/ 100 Assays(96 samples)

Measuring instrument：Spectrophotometer（340 nm）

Detection range：1-79 U/L

Elabscience® Glutathione-S-Transferase (GST) 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.

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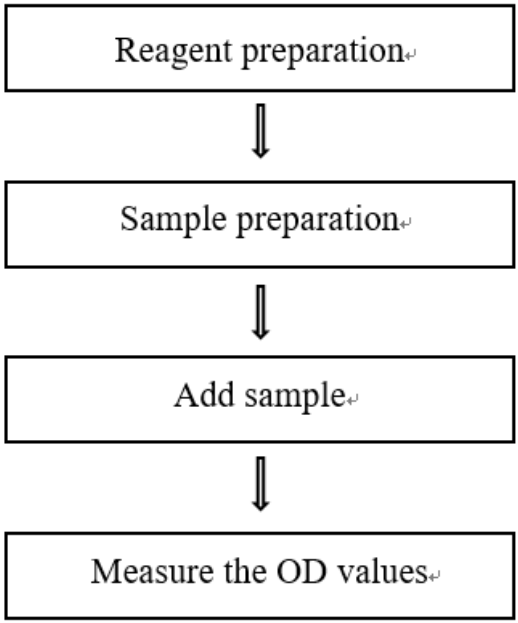
Assay summary



Intended use

This kit can be used to measure the Glutathione-S-Transferase (GSH-ST) activity in serum, plasma, tissue and cell samples.

Detection principle

GST can catalyze the binding of reduced glutathione (GSH) to dinitrobenzene (CDNB) and the product have an absorption peak at 340 nm. The activity of GSH-ST can be calculated by measuring the increasing rate of absorbance at 340 nm.

Kit components & storage

Item	Component	Size 1 (50 assays)	Size 2 (100 assays)	Storage
Reagent 1	Extracting Solution	60 mL ×1 vial	60 mL ×2 vials	2-8℃, 12 months
Reagent 2	Buffer Solution	50 mL ×1 vial	50 mL ×2 vials	2-8℃, 12 months
Reagent 3	Powder	Powder ×1 vial	Powder ×2 vials	2-8℃, 12 months

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:

Spectrophotometer (340 nm), Micropipettor, Incubator, Vortex mixer

Reagents:

Double distilled water

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② The preparation of powder application solution：
Dilute one vial of powder with 5 mL of double distilled water, mix well. Store at 2-8 ℃ for 3 days.

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).

Cells:

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 1×10^6 cells in 300 μL extracting solution with a ultrasonic cell disruptor 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
Human serum (plasma)	1
10% Rat liver tissue homogenate	150-200
10% Rat lung tissue homogenate	8-12
10% Rat kidney tissue homogenate	10-15
10% Plant tissue homogenate	1

Note: The diluent is double distilled water or extracting solution. For the dilution of other sample types, please do pretest to confirm the dilution factor

Operating steps

① Preheat the needed powder application solution, buffer solution and cuvette at 37 ℃ for 10 min. Set the spectrophotometer to 340 nm and set the spectrophotometer to zero with double distilled water.

② Blank tube: add 0.1 mL of extracting solution to a 2 mL EP tube.
Sample tube: add 0.1 mL of sample to a 2 mL EP tube.

③ Add 0.9 mL of buffer solution and 0.1mL of powder application solution to each tube, mix fully and record the time immediately. Measure the absorbance at 340 nm at 20 s (A_1), incubate at 37℃ and measure the absorbance at 340 nm at 320 s (A_2). Calculate the $\triangle A_{blank}$ or sample $=A_2-A_1$.

Calculation

The sample:

1. Serum (plasma) and other liquid sample:

Definition: The amount of GSH-ST in 1 mL of sample that catalyze the combination of 1 μmol of CDNB and GSH at 37 ℃ per minute is defined as 1 unit.

$$\text{GST activity (U/mL)} = \frac{\Delta A}{\varepsilon \times d} \times 10^6 \div t \times \frac{V_1}{V_2} \times f$$

2. Tissue and cells sample:

$$\text{GST activity (U/mgprot)} = \frac{\Delta A}{\varepsilon \times d} \times 10^6 \div t \times \frac{V_1}{V_2} \times f \div C_{pr}$$

[Note]

ΔA: $\Delta A_{Sample} - \Delta A_{Blank}$.

ε: molar extinction coefficient of the product, 9.6×10^3 L/mol/cm;

d: optical path of the cuvette, 1 cm;

10^6: 1 mol=10^6 μmol

V_1: the total volume of the reaction system, (1.1 mL = 0.0011 L);

V_2: the volume of sample added into the reaction system, 0.1 mL;

t: reaction time, 5 min;

C_{pr}: concentration of protein in sample, mgprot/mL;

f: dilution factor of sample before test.

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)	5.60	24.50	52.30
%CV	2.3	1.8	1.6

Inter-assay Precision

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	5.60	24.50	52.30
%CV	4.2	4.3	4.4

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 105%.

	Sample 1	Sample 2	Sample 3
Expected Conc.（U/L）	12.6	32.5	65
Observed Conc.（U/L）	13.0	33.8	70.2
recovery rate(%)	103	104	108

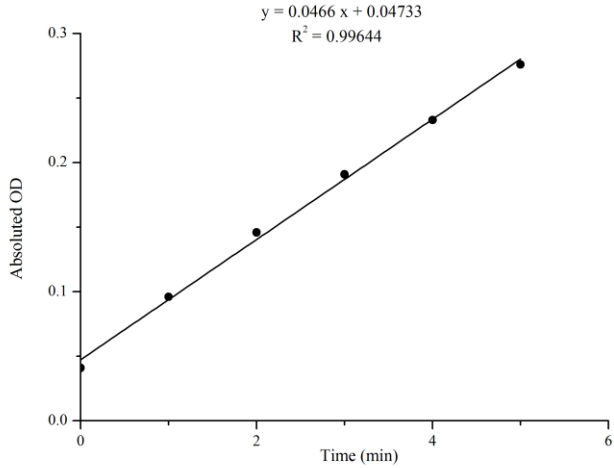
Sensitivity

The analytical sensitivity of the assay is 1 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:

Time (min)	**0**	**1**	**2**	**3**	**4**	**5**
Absoluted OD	0.041	0.096	0.146	0.191	0.233	0.276



Appendix II Example Analysis

Example analysis:

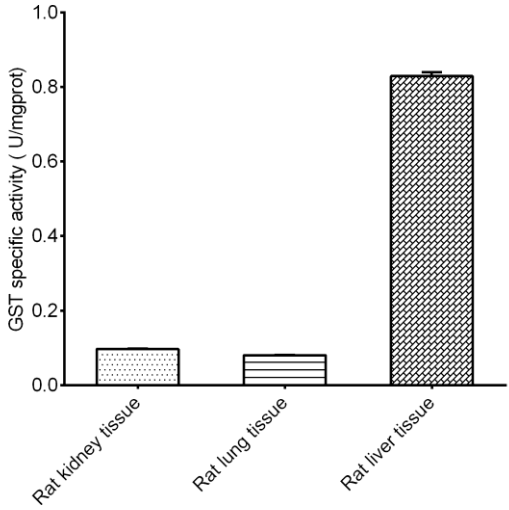
Dilute 10% rat kidney tissue homogenate with reagent 1 at the ratio of 1:14, take 0.1 mL of diluted sample and carry the assay according to the operation steps. The results are as follows:

The average OD value of the sample (A_1) is 0.464, the average OD value of the sample (A_2) is 0.729, the average OD value of the blank (A_1) is 0.414, the average OD value of the blank (A_2) is 0.437, the concentration of protein in sample is 8.51 mgprot/mL and the calculation result is:

$\Delta A = (0.729 - 0.464) - (0.437 - 0.414) = 0.242$

$$\text{GST activity (U/mgprot)} = \frac{0.242}{1\times 9.6\times 10^{3}} \times 10^{6} \div 5 \times \frac{0.0011}{0.1} \times 15 \div 8.51 = 0.098 \text{ U/mgprot}$$

Detect 10% rat kidney tissue homogenate (the concentration of protein is 8.51 mgprot/mL, dilute for 15 times), 10% rat lung tissue homogenate (the concentration of protein is 4.72 mgprot/mL, dilute for 8 times), 10% rat liver tissue homogenate (the concentration of protein is 13.74 mgprot/mL, dilute for 200 times) 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.