IMMUNOLOGICAL SCIENCES Product Data Sheet

CO-K800-M Glutathione-S-Transferase (GST) Activity Assay Kit (DTNB Method)

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

Method: Colorimetric method

Measuring instrument: Microplate reader

Sensitivity: 2.1 U/L

Detection range: 2.1-92.8 U/L

Average intra-assay CV (%): 1.8

Average inter-assay CV (%): 6.4

Average recovery rate (%): 105

- ▲ 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 be used to measure the GST activity in serum, plasma and animal tissue samples.

▲Background

Glutathione S-transferase is a kind of enzyme related to liver detoxification, which is often used as an indicator of liver injury. GST can resist the damage of endogenous and exogenous electronophilic substances, and plays an important role in the anti-tumor process.

▲Detection principle

GST can catalyze the binding of reduced glutathione (GSH) to dinitrobenzene (CDNB). The enzyme activity is indicated by measuring the substrate GSH binding rate with dinitrodiphenyl in unit time, the reaction of the rest of the GSH acts with disulfide double nitro benzoic acid (DTNB) to form yellow glucosinolates nitro benzoic acid anion (TNB), the concentration of which is determined to calculate the reduction of GSH. Thus, the activity of glutathione S-transferase (GST) was calculated indirectly by measuring the OD value at 412 nm.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Substrate	Powder × 1 vial	2-8℃ , 12 months
Reagent 2	Stock Diluent	12 mL × 1 vial	2-8℃ , 12 months
Reagent 3	Stop Solution	50 mL × 1 vial	2-8℃ , 12 months
Reagent 4	Phosphate	15 mL × 1 vial	2-8℃ , 12 months
Reagent 5	DTNB Solution	5 mL × 1 vial	2-8℃ , 12 months, shading light
Reagent 6	Standard	Powder × 1 vial	2-8℃ , 12 months
Reagent 7	Standard Stock Solution	3 mL × 1 vial	2-8℃ , 12 months
	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

Microplate reader (412 nm), Test tube, Micropipettor, Vortex mixer, Centrifuge, 37℃ incubator.

Reagents

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

▲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. During the color reaction, take the supernatant carefully after the incubation reaction to avoid take the precipitate.
2. Reaction time and operation time must be strictly controlled.

Pre-assay preparation

▲Reagent preparation

1. Bring all reagents to room temperature before use.
2. Preparation of reagent 1 working solution:
   Dissolve reagent 1 with 10 mL of reagent 2. Prepare the fresh solution before use and the prepared solution can be stored at 2-8℃ for 1 day.
3. Preparation of reagent 7 application solution:
   Mix the reagent 7 and double distilled water at a ratio of 1:9. Prepare the fresh solution before use and the prepared solution can be stored at 2-8℃ for 3 days.
4. Preparation of 1 mmol/L standard solution:
   Dissolve reagent 6 with 10 mL of reagent 7 application solution. Prepare the fresh solution before use and the prepared solution can be stored at 2-8℃ for 3 days.
5. Preparation of 250 μmol/L standard solution:
   Dilute 1 mmol/L standard solution and reagent 7 application solution at a ratio of 1:3. Prepare the fresh solution before use and the prepared solution can be stored at 2-8℃ for 3 days.

▲Sample preparation

1. Serum (Plasma): Detect the sample directly.

2. Tissue sample: Accurately weigh the tissue sample, add PBS (0.01 M, pH 7.4) according to the ratio of Weight (g): Volume (mL) =1:9. Mechanical homogenate the sample in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant.

▲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 (2.1-92.8 U/L).

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

Sample type	Dilution factor
Human plasma (serum)	1
Horse serum	1
Rat serum	1
Rabbit serum	1
Porcine serum	1
10% Rat kidney tissue homogenate	1
10% Rat brain tissue homogenate	1
10% Rat liver tissue homogenate	1
10% Rat spleen tissue homogenate	1
10% Rat lung tissue homogenate	1

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4).

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 operating steps

The preparation of standard curve

Dilute 250 μmol/L GSH standard with reagent 7 application solution to a serial concentration. The recommended dilution gradient is as follows: 0, 25, 75, 100, 125, 150, 200, 250 μmol/L. Reference is as follows:

Number	Standard concentrations (μmol/L)	250 μmol/L GSH Standard (μL)	Reagent 7 application solution (μL)
A	0	0	300
B	25	30	270
C	75	90	210
D	100	120	180
E	125	150	150
F	150	180	120
G	200	240	60
H	250	300	0

The measurement of samples

1. Enzymatic reaction

1) Control tube: take 60 μL of reagent 1 working sotution to a 1.5 mL EP tube.
   Sample tube: take 60 μL of reagent 1 working sotution and 20 μL of sample to a 1.5 mL EP tube.
2) Incubate at 37℃ for 30 min.
3) Take 400 μL of reagent 3 and 20 μL of sample to control tubes, mix fully.
   Take 400 μL of reagent 3 to sample tubes and mix fully.
4) Centrifuge at 3500 g for 10 min, take 100 μL of the supernatant for color reaction. (If there is precipitation in the supernatant, take the supernatant into a new EP tube and centrifuge again.)

2. Color reaction

1) Standard well: add 100 μL of standard solution with different concentrations into the corresponding wells.
   Control well: add 100 μL of control supernatant into the corresponding wells.
   Sample well: add 100 μL of sample supernatant into the corresponding wells.
2) Add 100 μL of reagent 4 and 25 μL of reagent 5 into each well.
3) Mix fully for 5 s with microplate reader and stand at room temperature for 5 min. Measure the OD values of each well at 412 nm with microplate reader.

▲Summary operation table

1. Enzymatic reaction

	Control tube	Sample tube
Reagent 1 working solution (μL)	60	60
Sample (μL)		20
Mix fully and incubate at 37℃ for 30 min.		
Reagent 3 (μL)	400	400
Sample (μL)	20	
Centrifuge at 3500 g for 10 min, take 100 μL of the supernatant for color reaction.		

2. Color reaction

	Standard well	Control well	Sample well
Standards with different concentrations (μL)	100		
Control supernatant (μL)		100	
Sample supernatant (μL)			100
Reagent 4 (μL)	100	100	100
Reagent 5 (μL)	25	25	25
Mix fully and stand at room temperature for 5 min. Measure the OD values at 412 nm.			

▲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. Serum (plasma) and other liquid sample

Definition: the enzyme amount of 1 μmol/L of GSH concentration decreased by 1 L of sample per minute at 37 ℃ in the reaction system is defined as 1

$$\text{GST activity (U/L)} = (\Delta A_{412} - b) \div a \div t \times 24 \times f$$

2. Tissue sample

Definition: the enzyme amount of 1 μmol/L of GSH concentration decreased by 1 g of tissue protein per minute at 37℃ in the reaction system is defined as 1 unit.

$$\text{GST activity (U/gprot)} = (\Delta A_{412} - b) \div a \div t \times 24 \times f \div C_{pr}$$

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_{412}: $OD_{Control} - OD_{Sample}$;

t: Enzymatic reaction time, 5 min;

24: Dilution factor of sample in the enzymatic reaction;

f: Dilution factor of sample before test;

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

Appendix I Data

▲Example analysis

Take 20 μL of human serum and carry the assay according to the operation table. The results are as follows:

Standard curve: y = 0.0026 x – 0.0017, the OD value of the sample is 0.411, the OD value of the control is 0.612, and the calculation result is:

GST activity (U/L)=(0.612-0.411+0.0017)÷0.0026÷30×24=62.4 U/L

For Research use only