Technical Manual

Glutathione-S-Transferase (GST) Colorimetric Assay Kit (DTNB method)

- Catalogue Code: MAES0197
- Size: 96T
- Research Use Only

1. Key Features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

2.1-92.8 U/L

Sensitivity:

2.1 U/L

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. 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.

3. Intended Use

This kit can be used to measure the GST activity in serum, plasma and animal tissue samples.

4. 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.

5. Kit Components & Storage

Item	Specification	Storage
Substrate	Lyophilized × 1 vial	2-8°C, 6 months
Stock Diluent	12 mL × 1 vial	2-8°C, 6 months
Stop Solution	50 mL × 1 vial	2-8°C, 6 months
Phosphate	15 mL × 1 vial	2-8°C, 6 months
DTNB Solution	5 mL × 1 vial	2-8°C, 6 months, avoid direct sunlight
Standard	Lyophilized × 1 vial	2-8°C, 6 months
Standard Stock Solution	3 mL × 1 vial	2-8°C, 6 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- incubator
- Vortex mixer
- Centrifuge
- Microplate Reader (412 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- 60% Ethanol

6. Assay Notes:

1. During the color reaction, take the supernatant carefully after the incubation reaction to prevent take the precipitate.

2. Reaction time and operation time must be strictly controlled.

7. Reagent Preparation:

1. Bring all reagents to room temperature before use.

2. Preparation of **substrate working solution**: Dissolve substrate with 10 mL of stock diluent. Prepare the fresh solution before use and the prepared solution can be stored at 2-8°C for 1 day.

3. Preparation of **standard stock application solution**: Mix the standard stock solution 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°C for 3 days.

4. Preparation of **standard solution (1 mmol/L)**: Dissolve standard with 10 mL of standard stock application solution. Prepare the fresh solution before use and the prepared solution can be stored at 2-8°C for 3 days.

5. Preparation of **standard solution (250 μmol/L)**: Dilute 1 mmol/L standard solution and standard stock application solution at a ratio of 1:3. Prepare the fresh solution before use and the prepared solution can be stored at 2-8°C for 3 days.

8. Sample Preparation

1. Serum sample:

Fresh blood should be incubated at 25°C for 30 min to clot the blood. Centrifuge the sample at 2000 g for 15 min at 4°C. Take the serum (which is the upper light yellow clarified liquid layer) and preserve on ice before detection. If not detected on the same day, the serum can be stored at -80°C for a month.

2. Plasma sample:
Place the fresh blood sample into a tube of anticoagulant and centrifuge at 700-1000g for 10 min at 4°C. Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) and preserve on ice before detection. If not detected on the same day, the plasma can be stored at -80°C for a month.

3. Tissue sample:
Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C. Use filter paper to absorb excess water and weigh. Homogenize at the ratio of the volume of PBS (0.01 M, pH 7.4) (2-8°C) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4°C. Take the supernatant and preserve on ice before detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

Sample Notes:
The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:
Large variances in results may be seen when performing pre-experiments. 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).

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 412 nm

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.

10. Operation Steps

The preparation of standard curve

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

The measurement of samples

Enzymatic reaction

1. **Control tube:** take 60 μL of substrate working sotution to a 1.5 mL EP tube.
   Sample tube: take 60 μL of substrate working sotution and 20 μL of sample to a 1.5 mL EP tube.

2. Incubate at 37°C for 30 min.

3. Take 400 μL of stop solution and 20 μL of sample to control tubes, mix fully.

4. Take 400 μL of stop solution to sample tubes and mix fully

5. 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).

Color reaction

1. **Standard well:** add 100 µL of standard solution with different concentrations into the corresponding wells.
   Control well: add 100 µL of standard solution with different concentrations into the corresponding wells.
   Sample well: add 100 µL of sample supernatant into the corresponding wells.

2. Add 100 µL of phosphate and 25 µL of DTNB solution into each well.

3. Mix fully for 5s with microplate reader and stand at room temperature for 5 min. Measure the OD values of each well at 412 nm with microplate reader.

Operation Table

Enzymatic reaction

	Control tube	Sample tube
Substrate working solution (µL)	60	60
Sample (µL)		20
Mix fully and incubate at 37°C for 30 min.		
Stop Solution (µL)	400	400
Sample (µL)	20	
Centrifuge at 3500 g for 10 min, take 100 µL of the supernatant for color reaction.		

Note: If there is precipitation in the supernatant, take the supernatant into a new EP tube and centrifuge again.

Color reaction

	Standard well	Sample well	Control well
Standards solution with different concentrations (µL)	100		
Control supernatant (µL)		100	
Sample supernatant (µL)			100
Phosphate (µL)	100	100	100
DTNB Solution (µL)	25	25	25
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.			

11. Calculations

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°C in the reaction system is defined as 1 unit.

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

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°C in the reaction system is defined as 1 unit.

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

y: $\triangle_{Standard} - \triangle_{Blank}$ ($\triangle_{Blank}$ 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.
C_{pr}: Concentration of protein in sample, mgprot/mL.
f: Dilution factor of sample before test.

12. Performance Characteristics

Detection Range	2.1-92.8 U/L
Sensitivity	2.1 U/L
Average recovery rate (%)	105
Average inter-assay CV (%)	6.4
Average intra-assay CV (%)	1.8

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:

$$\text{GST activity (U/L)} = (0.612 - 0.411 + 0.0017) \div 0.0026 \div 30 \times 24$$

$$= 62.4 \text{ U/L}$$

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Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching 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.

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

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