Glutathione-S-transferase assay kit

(Cat/No.: BC045 Size: 100T/48S)

1. Test principle (Colorimetric method)

$$(GSH+C_6H_3(NO_2)_2Cl) \xrightarrow{GST} C_6H_3(NO_2)_2GS+H^++Cl^-$$

GST catalyzes the binding of reduced glutathione (GSH) to 1-chloro-2,4-dinitrobenzene (CDNB substrate). Within a given reaction time, its activity is linearly related to the change in substrate concentration before and after the reaction.

This experiment uses the concentration of GSH to indicate the activity of GST; the greater the decrease in GSH (substrate) concentration, the greater the GST activity.

2.Composition and preparation (The kit is valid 6 months)

<table>
<tr><td></td><td>Reagent composition</td><td>Specification</td><td>Storage conditions</td></tr>
<tr><td rowspan="3">Reagent 1</td><td>Powder A</td><td>Powder x 1 vial</td><td>Store at 2～8℃</td></tr>
<tr><td colspan="3">Preparation of Solution A: Dissolve each vial of powder in 1 mL of anhydrous ethanol until fully dissolved. It can be stored at 2–8°C for one month (discard if the color darkens).</td></tr>
<tr><td>Liquid B</td><td>60mL×1 bottle</td><td>Store at 2～8℃</td></tr>
<tr><td colspan="4">Preparation of reagent 1 application solution: Prepare solution A : solution B in a ratio of 1 : 59. Prepare fresh solution immediately before use.</td></tr>
<tr><td rowspan="2">Reagent 2</td><td>Powder A</td><td>Powder x 1 bottle</td><td>Store at 2～8℃</td></tr>
<tr><td>Liquid B</td><td>50mL×1 bottle</td><td>Store at 2～8℃</td></tr>
<tr><td colspan="4">Preparation of Reagent 2 Solution: Add 172 mL of distilled water to powder A, heat to 90–100 °C and stir until fully dissolved. Then mix solutions A and B and stir thoroughly. This mixture is a supersaturated solution and should be stored at room temperature. If crystallization occurs, use the supernatant directly for the experiment.</td></tr>
<tr><td>Reagent 3</td><td>White powder</td><td>Powder x 1 bottle</td><td>Store at 2～8℃</td></tr>
<tr><td colspan="4">Preparation of reagent 3 application solution: Add 200mL of distilled water, dissolve at room temperature and set aside (the provided plastic bottle can be used for preparation), store at room temperature.</td></tr>
<tr><td>Reagent 4</td><td>pale yellow powder</td><td>Powder x 1 vial</td><td>Store at 2～8℃</td></tr>
<tr><td colspan="4">Preparation of reagent 4 application solution: Add 50 mL of distilled water, dissolve at room temperature, and store in the dark at 2–8 °C.</td></tr>
<tr><td>Reagent 5</td><td>GSH Standard Products</td><td>3.07mg powder x 3 vials</td><td>Store at 2～8℃</td></tr>
<tr><td>Reagent 6</td><td>GSH standard solvent stock solution</td><td>10mL×1 bottle</td><td>Store at 2～8℃</td></tr>
<tr><td colspan="4">Preparation of GSH standard solvent application solution: GSH standard solvent stock solution: dilute 10 times with distilled water at a ratio of 1:9 .</td></tr>
<tr><td colspan="4">Preparation of 1 mmol/L GSH standard solution: Add one vial of GSH standard to 10 mL of standard solvent working solution, mix well and dissolve. It can be stored at 4℃ for 1-2 weeks.</td></tr>
<tr><td colspan="4">Preparation of 20 mol/L GSH standard solution: Take 0.1 mL of 1 mmol/L GSH standard solution and add 4.9 mL of standard solvent working solution. Prepare fresh before use.</td></tr>
</table>

Matrix solution preparation: Mix reagent 1 working solution with 1 mmol/L LSH standard solution at a 1:1 ratio, and Prepare as needed.

3.Scope of application

This kit can be used to test samples such as serum, tissue, and cultured cells.

4. Required instruments

Visible spectrophotometer and 1cm path length cuvette (or microplate reader (400~415nm) and 96-well plate), 37℃ constant temperature water bath, electric furnace, low speed centrifuge, protein assay reagents .

5. Sample pretreatment

1.Serum or plasma: can be used directly.

2.Tissue sample pretreatment: Weigh the animal tissue to be tested, add 9 times the volume of physiological saline at a ratio of weight (g):volume (mL) = 1:9, homogenize at low temperature (0-4℃), centrifuge at 3500 rpm for 10 minutes, and take the supernatant for testing (the protein concentration of the prepared homogenate supernatant needs to be determined, and the protein assay kit BC016 is available in our company).

3.Cell sample pretreatment: (Adherent cells) Scrape off the cells with a cell scraper and isotonic PBS or digest them with trypsin (rinse with 0.5-1 mL isotonic PBS after digestion). Transfer the cell suspension to another centrifuge tube and centrifuge at 1000 rpm for 10 minutes. Discard the supernatant and keep the cell pellet.

Wash the cells 1-2 times with isotonic PBS, centrifuge at 1000 rpm for 10 minutes, discard the supernatant, and keep the cell pellet (if not to be measured immediately, it can be stored directly in a -20℃ or -80℃ refrigerator and can be used within 3 months). Add 0.2-0.3 mL of homogenization medium (physiological saline is recommended) to the cell pellet. (After adding the homogenization medium, gently mix the cell solution to make it homogeneous, and take a small amount for cell counting; if protein can be measured after disruption, cell counting is not necessary). Sonicate the cells under ice-water bath conditions (power: 200-300W, 3-5 seconds/time, 15-second interval, repeat for 3-5 minutes) or homogenize manually. If the prepared homogenate is relatively homogeneous, it can be measured directly without centrifugation. Alternatively, lysis buffer (Triton X-100, 1-2% concentration, 0.1 mL, lysis on ice for 30-40 minutes) can be used. The lysed liquid can be directly analyzed without centrifugation. [Note]: It is recommended to have a cell count of more than 1 million (the higher the number, the better the measurement results). The lysed liquid can be observed under a microscope to see if the cells are completely lysed.

4.Preparation of hemolyzed blood: Take 20 µL of heparin-anticoagulated whole blood, dilute it to 1 mL (50-fold dilution) with distilled water, mix well, and let it stand at 4°C for 5 minutes before measurement. The prepared hemolyzed blood should be measured within 1 hour, otherwise it will affect enzyme activity; if the anticoagulated whole blood cannot be measured on the same day, it can be stored in a refrigerator at 4°C, and the enzyme

activity will not change much within 2-3 days.

6. Operating steps

1.Serum (plasma), tissue, and cell sample handling:

Step 1: Enzymatic reaction

	Measurement tube	control tube
matrix solution(mL)	0.3	0.3
sample(mL)	0.1	
Mix well and incubate in a 37°C water bath for 30 minutes.		
Reagent 2 Application Solution(mL)	2	2
sample(mL)		0.1
Mix well, centrifuge at 4000 rpm for 10 minutes, and use the supernatant for colorimetric reaction.		

Step 2: Colorimetric Reaction

	Blank tube	Standard pipe	Measurement tube	control tube
GSH Standard Solvent Application Solution(mL)	2			
20μmol/LGSH standard solution(mL)		2		
Supernatant (mL)			2	2
Reagent 3 Application Solution(mL)	2	2	2	2
Reagent 4 Application Solution(mL)	0.5	0.5	0.5	0.5
Mix well, let stand at room temperature for 15 minutes, and read the absorbance A of each tube at a spectrophotometer with a wavelength of 412 nm using distilled water with a 1 cm optical path (or pipette 200 μL of reaction solution into each well of a 96-well plate and read the absorbance at 412 nm using an ELISA reader).				

Note: If the A control – A measurement value is greater than 0.3, dilute the sample and test again, or conduct a pre-test to control the A control – A measurement value between 0.1 and 0.2 (the microplate reader reading can be around 0.1, and when the enzyme activity is low, the value can be greater than 0.02).

Unit definition and calculation formula

Definition of a unit of liquid sample such as serum (plasma): One unit of enzyme activity (U) is defined as the
reduction of the GSH concentration in the reaction system by 1 mol/L after reacting each milliliter of serum (plasma) at 37°C for 1 minute, excluding non-enzymatic reactions.

Definition of tissue and cell sample unit: One enzyme activity unit (U) is defined as the amount of enzyme

corresponding to each milligram of tissue protein that reduces the concentration of GSH in the reaction system by 1 mol/ L per minute at 37°C after deducting non-enzymatic reactions.

Formula for calculating serum (plasma) and other liquid samples:

Formula for calculating serum (plasma) and other liquid samples:

$$\text{GST activity (U/mL)} = \frac{A_{control} - A_{sample}}{A_{standard} - A_{blank}} \times C_{standard} \times N \div T \div V_{sample} \times N_{dilution}$$

Formulas for calculating tissue and cell samples:

$$\text{GST activity (U/mgprot)} = \frac{A_{control} - A_{sample}}{A_{standard} - A_{blank}} \times C_{standard} \times N \div T \div (V_{sample} \times C_{prot})$$

In the above formula:

C standard: Standard concentration, 20 mol/ L;

N: Reaction system dilution factor, 6 (2.4 mL / 0.4 mL, fixed value);

T: Enzymatic reaction time, 30 minutes;

V sample: Sample volume, 0.1 mL;

N sample: Sample dilution factor before testing;

Cpr: Tissue homogenate protein concentration, mgprot/ mL (prot refers to protein);

7. Calculation Example

Example 1: 0.1 mL of tilapia serum was used to detect GSH-ST activity. The absorbance values were: test tube 0.436, control tube 0.485, standard tube 0.160, and blank tube 0.037. The calculations are as follows:

$$\text{Serum GST activity (U/mL)} = \frac{0.485-0.436}{0.160-0.037} \times 20 \times 6 \div 30 \div 0.1$$

$$= 15.93\ \text{U/mL}$$

Example 2: 0.1 mL of undiluted porcine serum was used to detect the activity of GSH-ST. The absorbance values

were: test tube 0.403, control tube 0.475, standard tube 0.160, and blank tube 0.037. The calculations are as follows:

$$\text{Serum GST activity (U/mL)} = \frac{0.475-0.403}{0.160-0.037} \times 20 \times 6 \div 30 \div 0.1$$

$$= 23.41\ \text{U/mL}$$

Example 3: A 10% homogenate of mouse liver tissue was diluted 5 times with physiological saline to a 2%

concentration. The procedure was performed according to the control table. The absorbance values were: test tube 0.251, control tube 0.455, standard tube 0.160, and blank tube 0.037. The protein concentration of the 2%

homogenate was 1.15 mg prot/mL. The calculations are as follows:

$$\text{Tissue GST activity (U/mgprot)} = \frac{0.455-0.251}{0.160-0.037} \times 20 \times 6 \div 30 \div (0.1 \times 1.15)$$

$$= 57.69\ \text{U/mgprot}$$

2. Blood lysate (whole blood 1:49 plus distilled water) sample handling:

Step 1: Enzymatic reaction

	Measurement tube	control tube
matrix solution (mL)	0.2	0.2
Hemolysis to be tested (mL)	0.2	
Mix well and incubate in a 37°C water bath for 30 minutes.		
Reagent 2 Application Solution (mL)	2	2
Hemolysis to be tested (mL)		0.2
Mix well, centrifuge at 4000 rpm for 10 minutes, and use the supernatant for color development.		

Step 2: Colorimetric Reaction

	Blank tube	Standard pipe	Measurement tube	control tube
GSH Standard Solvent Application Solution (mL)	2			
20μmol/ LGSHstandard solutions (mL)		2		
Supernatant (mL)			2	2

Reagent 3 Application Solution (mL)	2	2	2	2
Reagent 4 Application Solution (mL)	0.5	0.5	0.5	0.5
Mix well and let stand at room temperature for 15 minutes. Use a spectrophotometer with a 1cm optical path and distilled water to zero the reading at 412nm to read the absorbance value A of each tube (or take 200μL of reaction solution from each tube, add it to a 96-well plate, and read the absorbance at 412nm using a microplate reader).				

3. Unit definition and calculation formula

Unit definition: one unit of enzyme activity (U) is defined as the reduction of the GSH concentration in the reaction

system by 1 μmol/L per minute at 37。C, excluding non-enzymatic reactions.

Calculation formula:

$$\text{Whole blood GST activity (U/mL)} = \frac{A_{control} - A_{sample}}{A_{standard} - A_{blank}} \times C_{standard} \times N \div T \div V_{whole\ blood}$$

V. Whole Blood: 0.2 mL of a 50-fold diluted hemolysate is equivalent to 0.004 mL of whole blood (0.2 mL ÷ 50 = 0.004 mL);

C. Standard: Standard concentration, 20μmol/L;

N: Reaction system dilution factor, 6 (2.4 mL / 0.4 mL, fixed value);

T: Enzyme reaction time, 30 minutes;

4. Calculation Example

Take 20μL of whole blood to prepare a 1:49 hemolysate. Take 0.2 mL of the hemolysate to test GST activity. The

absorbance values of the test tube, control tube, standard tube, and blank tube were 0.231, 0.280, 0.160, and 0.037, respectively.

$$\text{Whole blood GST activity (U/mL whole blood)} = \frac{0.280-0.231}{0.160-0.037} \times 20 \times 6 \div 0.004 \div 30$$

$$= 398.37\ \text{U/mL whole blood}$$

8. Significance of measurement

Glutathione S-transferase (GST) is a class of enzymes involved in liver detoxification. It is present in large quantities in hepatocytes. Therefore, when hepatocytes are damaged, GSH-ST is often released into the blood very early. The increase in blood GSH-ST often precedes that of alanine aminotransferase (SGPT) and aspartate aminotransferase (SGOT). Thus, the increase in GSH-ST can be used as a sensitive indicator of liver damage.

Glutathione S-transferase is widely found in various tissues of mammals. It catalyzes the binding of glutathione (GSH) to the electrophilic groups of chemical substances, ultimately forming thioether amino acids that are excreted from the body, playing an important role in the body's detoxification function.

GSH-ST has a dual function of eliminating peroxides and detoxification in the body. Under conditions of low glutathione peroxidase (GSH-PX) activity, GST only has the function of clearing lipid peroxides (LPO) in the body.

9. Precautions

1) When preparing dimethyl methacrylate (DMMA) reagent, it is best to dissolve it by direct heating. Indirect heating methods such as water bath or oven may not be able to dissolve it.

2) Heparin-anticoagulated whole blood should not be stored in a refrigerator for more than 3 days, while plasma and tissue blocks can be stored at -20°C for more than one month.

3) The supernatant was extracted and tested on the same day.

4) This kit is for research and laboratory use only.