

Glutathione S-transferase (GST) Activity Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer/Microplate Reader

Cat Number: BC0355

Size: 100T/96S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation Condition
Reagent I	Liquid 110 mL×1	2-8°C
Reagent II	Liquid 22 mL×1	2-8°C
Reagent III	Powder×1	2-8°C

Solution Preparation:

1. Reagent III: Dissolve with 3 mL of distilled water before use. Store at 2-8°C for 4 weeks.

Product Description

Glutathione S-transferase (GST) is a family of proteins with many physiological functions, which mainly exists in the cytoplasm. GST is an important part of detoxification enzyme system in the body. It mainly catalyzes various chemical substances and their metabolites to covalent bond with the sulfhydryl group of GSH. So that electrophilic compounds become hydrophilic substances, which are easy to be excreted from bile or urine, so as to degrade various potentially toxic substances in the body and expel them out of the body. Therefore, GST plays an important biological role in protecting cells from electrophilic compounds. In addition, because GST has the activity of GSH-Px, it is also called non-se GSH-px and has the function of repairing macromolecular such as DNA and protein damaged by oxidation. Note that GST-catalyzed reactions reduce GSH content but do not increase GSSG content.

GST catalyzed the binding of GSH with CDNB, and the light absorption peak wavelength of the binding product is 340 nm. Calculate the GST activity by measuring the absorbance rising rate at the wavelength of 340 nm.

Reagents and Equipment Required but Not Provided

Spectrophotometer/microplate reader, refrigerated centrifuge, water bath/constant temperature incubator, micro quartz cuvette/96 well UV flat-bottom plate, mortar/homogenizer, transferpettor, ice and distilled water.

Procedure

I. Extraction of crude enzyme solution:

1. Tissue:

According to the tissue weight (g): Reagent I volume (mL) is 1:5-10 (it is recommended that add 1 mL of Reagent I to 0.1 g of tissue) for ice bath homogenization. Centrifuge at 8000 ×g for 10 minutes at 4°C to

remove insoluble materials and take the supernatant on ice for testing.

2. Bacteria or cells:

According to the number of bacteria or cells (10^4): Reagent I volume (mL) is the proportion of 500~1000: 1 (it is recommended that add 1 mL of Reagent I to 5 million bacteria or cells), and

break the bacteria or

cells by ultrasound (placed on ice, ultrasonic power 300W, working time 3 seconds, interval 7 seconds, repeat for 18 times). Centrifuge at 8000 ×g for 10 minutes at 4°C to remove insoluble materials and take the supernatant on ice for testing.

3. Serum (plasma): Detect sample directly.

II. Procedure

1. Preheat the spectrophotometer/microplate reader for more than 30 minutes, adjust the wavelength to 340 nm and adjust the zero with distilled water.

2. Keep the Reagent II warm at 37°C while in use.

3. Blank tube: Take a micro quartz cuvette or 96 well UV flat-bottom plate, add 20 μL of Reagent I, 180 μL of Reagent II and 20 μL of Reagent III. Mix thoroughly and timing, detect the absorbance at 340 nm at the time of 10 seconds record as A1. Then place cuvette with the reaction solution in a 37°C water bath for 5 minutes. Take it out and wipe it clean, immediately measure the absorbance of final reaction which record as A2.

4. Test Tube: Take a micro quartz cuvette or 96 well UV flat-bottom plate, add 20 μL of supernatant, 180 μL of Reagent II and 20 μL of Reagent III. Mix thoroughly and timing, detect the absorbance at 340 nm at the time of 10 seconds record as A3. Then place cuvette with the reaction solution in a 37°C water bath for 5 minutes. Take it out and wipe it clean, immediately measure the absorbance of final reaction which record as A4.

III. Calculation of GST activity

A. The formula for the determination of micro cuvette is as follows:

1) Calculate by sample protein concentration

Active unit (U) Definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the combination of 1 μmol of CDNB with GSH in the reaction system at 37°C per minute every milligram protein.

$$\text{GST (U/mg prot)} = [(A4-A3)-(A2-A1)] \div (\epsilon \times d) \times 10^6 \times V_{rv} \div (C_{pr} \times V_{rs}) \div T$$

$$= 0.23 \times [(A4-A3)-(A2-A1)] \div C_{pr}$$

2) Calculate by sample fresh weight

Active unit (U) Definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the combination of 1 μmol of CDNB with GSH in the reaction system at 37°C per minute every gram tissue sample.

$$\text{GST (U/g fresh weight)} = [(A4-A3)-(A2-A1)] \div (\epsilon \times d) \times 10^6 \times V_{rv} \div (V_{rs} \div V_{s1} \times W) \div T$$

$$= 0.23 \times [(A4-A3)-(A2-A1)] \div W$$

3) Calculate by the number of cells

Active unit (U) Definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the combination of 1 μmol of CDNB with GSH in the reaction system at 37°C per minute every 10⁴ cells.

$$\text{GST (U/10}^4 \text{ cell)} = [(A4-A3)-(A2-A1)] \div (\epsilon \times d) \times 10^6 \times V_{rv} \div (500 \times V_{rs} \div V_{s1}) \div T$$

$$= 0.23 \times [(A4-A3)-(A2-A1)] \div 500$$

4) Calculate by liquid volume

Active unit (U) Definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the combination of 1 μmol of CDNB with GSH in the reaction system at 37°C per minute every milliliter liquid sample.

$$\text{GST (U/mL)} = [(A4-A3)-(A2-A1)] \div (\epsilon \times d) \times 10^6 \times V_{rv} \div V_{rs} \div T$$

$$= 0.23 \times [(A4-A3)-(A2-A1)]$$

ϵ : Molar extinction coefficient for the product, 9.6×10^3 L/mol/cm.

d: Light diameter of the cuvette, 1 cm;

10^6 : 1 mol = 1×10^6 μ mol;

V_{rv} : The total volume of the reaction system, 220 μ L = 2.2×10^{-4} L;

C_{pr} : The protein concentration of the supernatant (mg/mL) needs to be determined in addition. (It is recommended to use (It is recommended to use #PC0020 BCA Protein Assay Kit)

V_{rs} : Add supernatant liquid volume into the reaction system, 20 μ L = 0.02 mL;

T: Reaction time, 5 minutes;

W: Sample fresh weight, g;

500: 5 million cells or bacteria;

V_{s1} : Volume of solution I, 1 mL.

B. The formula for the determination of 96 well UV plate is as follows:

1) Calculate by sample protein concentration

Active unit (U) Definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the combination of 1 μ mol of CDNB with GSH in the reaction system at 37°C per minute every milligram protein.

$$\text{GST (U/mg prot)} = [(A4-A3)-(A2-A1)] \div (\epsilon \times d) \times 10^6 \times V_{rv} \div (C_{pr} \times V_{rs}) \div T$$

$$= 0.38 \times [(A4-A3)-(A2-A1)] \div C_{pr}$$

2) Calculate by sample fresh weight

Active unit (U) Definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the combination of 1 μ mol of CDNB with GSH in the reaction system at 37°C per minute every gram tissue sample.

$$\text{GST (U/g fresh weight)} = [(A4-A3)-(A2-A1)] \div (\epsilon \times d) \times 10^6 \times V_{rv} \div V_{rs} \div V_{s1} \times W \div T$$

$$= 0.38 \times [(A4-A3)-(A2-A1)] \div W$$

3) Calculate by the number of cells

Active unit (U) Definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the combination of 1 μ mol of CDNB with GSH in the reaction system at 37°C per minute every 10⁴ cells.

$$\text{GST (U/10}^4 \text{ cell)} = [(A4-A3)-(A2-A1)] \div (\epsilon \times d) \times 10^6 \times V_{rv} \div (500 \times V_{rs} \div V_{s1}) \div T$$

$$= 0.38 \times [(A4-A3)-(A2-A1)] \div 500$$

4) Calculate by liquid volume

Active unit (U) Definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the combination of 1 μ mol of CDNB with GSH in the reaction system at 37°C per minute every milliliter liquid sample.

$$\text{GST (U/mL)} = [(A4-A3)-(A2-A1)] \div (\epsilon \times d) \times 10^6 \times V_{rv} \div V_{rs} \div T$$

$$= 0.38 \times [(A4-A3)-(A2-A1)]$$

ϵ : Molar extinction coefficient for the product, 9.6×10^3 L/mol/cm.

d: Light diameter of the cuvette, 0.6 cm;

10^6 : $1 \text{ mol} = 1 \times 10^6 \mu\text{mol}$;

Vrv: The total volume of the reaction system, $220 \mu\text{L} = 2.2 \times 10^{-4} \text{ L}$;

Cpr: The protein concentration of the supernatant(mg/mL) needs to be determined in addition. (It is

recommended to use #PC0020 BCA Protein Assay Kit);

Vrs: Add supernatant liquid volume into the reaction system, $20 \mu\text{L} = 0.02 \text{ mL}$;

T: Reaction time, 5 minutes;

W: Sample fresh weight, g;

Vs₁: Volume of solution I, 1 mL;

500: 5 million cells or bacteria.

Note:

1. Sample preparation and other processes need to be operated on the ice, and enzyme activity must be measured on the same day.
2. For cell sample test, keep cell amount between 3-5 million. The extraction of GST in cells can be followed by grinding or ultrasonic treatment with Reagent I, but not treated with cell lysate.
3. If the absorbance of the sample is greater than 1, dilute the sample with distilled water, and calculate result multiplied by dilution ratio.
4. Reaction temperature could infect determination result, general specie samples operated at 37°C .

Experimental Examples:

1. Take 0.1 g of rose and add 1 mL of Reagent I to conduct ice bath homogenization, centrifugation at 4°C and 8000g, for 10min, clean it, dilute 50 times and put it on ice for measurement. According to the measurement procedure with micro quartz cuvette, calculate the $\Delta A_T = A_4 - A_3 = 0.7046 - 0.62 = 0.0846$, $\Delta A_B = A_2 - A_1 = 0.5902 - 0.5207 = 0.0695$, and calculate according to the sample quality:

$\text{GST (U/g mass)} = 0.23 \times [(A_4 - A_3) - (A_2 - A_1)] \div W \times 50$ (dilution multiple) = 1.737 U/g mass.

2. Take 0.1 g of lung and add 1 mL of Reagent I to conduct ice bath homogenization, centrifugation at 4°C and 8000g for 10 min, clean it up, dilute 50 times and put it on ice for measurement. According to the measurement procedure, calculate the $\Delta A_T = A_4 - A_3 = 0.9402 - 0.5059 = 0.4343$, $\Delta A_B = A_2 - A_1 = 0.5902 - 0.5207 = 0.0695$, calculated according to the sample quality:

$\text{GST (U/g mass)} = 0.23 \times [(A_4 - A_3) - (A_2 - A_1)] \div W \times 50$ (dilution multiple) = 41.952 U/g mass.

Recent Product Citations:

[1] Xu J, Chu T, Yu T, Li N, Wang C, Li C, Zhang Y, Meng H, Nie G. Design of Diselenide-Bridged Hyaluronic Acid Nano-antioxidant for Efficient ROS Scavenging to Relieve Colitis. ACS Nano. 2022 Aug 23;16(8):13037-13048. doi: 10.1021/acsnano.2c05558. Epub 2022 Jul 21. PMID: 35861614.

References:

[1] Santillán-Sidón P, Pérez-Morales R, Anguiano G. et al. Glutathione S-transferase activity and genetic polymorphisms associated with exposure to organochloride pesticides in Todos Santos, BCS, Mexico: a preliminary study [J]. Environmental Science and Pollution Research, 2020, 27(34): 43223-43232.

Related Products:

BC1170/BC1175 Reduced Glutathione(GSH) Content Assay Kit
BC1180/BC1185 Oxidized Glutathione(GSSG) Content Assay Kit
BC1190/BC1195 Glutathione Peroxidase(GPX) Activity Assay Kit
BC1160/BC1165 Glutathione Reductases(GR) Activity Assay Kit