Glutathione S-transferase(GST) Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Catalog Number: BC0350

Size: 50T/48S

Components:

Reagent I: Liquid 50 mL×1. Storage at 4℃.

Reagent II: Liquid 45 mL×1. Storage at 4℃.

Reagent III: Powder×1. Storage at 4℃. Dissolve with 5 mL of distilled water before use.

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, refrigerated centrifuge, water bath, transferpettor, 1 mL quartz cuvette, mortar/homogenizer, 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℃ 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℃ to remove insoluble materials and take the supernatant on ice for testing.

3. Serum (plasma):

Detect sample directly.

II. Procedure

1. Preheat the spectrophotometer for more than 30 minutes, adjust the wavelength to 340 nm and adjust the zero with distilled water.
2. Keep the Reagent II and Reagent III warm at 25℃ (general species) or 37℃ (mammals) while in use.
3. Blank tube: Take a 1 mL quartz cuvette, add 100 μL of Reagent I, 900 μL of Reagent II and 100 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℃ (mammal) or 25℃ (general species) 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 1 mL quartz cuvette, add 100 μL of supernatant, 900 μL of Reagent II and 100 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℃ (mammal) or 25℃ (general species) 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

1. Calculate by sample protein concentration

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℃ or 25℃ per minute every milligram protein.

$$GST(U/mg\ prot) = [(A4-A3)-(A2-A1)] \div (\varepsilon \times d) \times 10^6 \times Vrv \div (Cpr \times Vrs) \div T$$
$$= 0.23 \times [(A4-A3)-(A2-A1)] \div Cpr$$

2. Calculate by fresh sample weight

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℃ or 25℃ per minute every gram tissue sample.

$$GST(U/g\ fresh\ weight) = [(A4-A3)-(A2-A1)] \div (\varepsilon \times d) \times 10^6 \times Vrv \div (Vrs \div Vs_1 \times W) \div T$$
$$= 0.23 \times [(A4-A3)-(A2-A1)] \div W$$

3. Calculate by cell amount

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℃ or 25℃ per minute every 10^4 cells.

$$GST(U/10^4\ cell) = [(A4-A3)-(A2-A1)] \div (\varepsilon \times d) \times 10^6 \times Vrv \div (500 \times Vrs \div Vs_1) \div T$$
$$= 0.23 \times [(A4-A3)-(A2-A1)] \div 500$$

4. Calculate by liquid volume

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℃ or 25℃ per minute every millilter liquid sample.

GST (U/mL)= [(A4-A3)-(A2-A1)]÷(ε×d)×10^6×Vrv ÷Vrs÷T

=0.23×[(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 μmol;

Vrv: Total volume of the reaction system, 1100 μL=1.1×10^{-4} L;

Cpr: Supernatant protein concentration (mg/mL).

Vrs: Add supernatant liquid volume into the reaction system, 100 μL =0.1 mL;

T: Reaction time, 5 minutes;

W: Sample fresh weight, g;

Vs_1: Volume of Reagent I, 1 mL.

Note:

1. Sample preparation processes should 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 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 25℃ and mammal samples at 37℃.

Experimental Examples：

1. Take 0.1 g of rose, add 1 mL of Reagent I, homogenize in ice bath, centrifuge at 4℃ and 8000g for 10min, take the supernatant, dilute 50 times, put it on ice for testing, operate according to the determination steps, and calculate ΔA_T = A4-A3 = 0.647-0.587 = 0.06, ΔA_B= A2-A1 = 0.591-0.539 = 0.052

GST (U/g mass) = 0.23 × [(A4-A3) - (A2-A1)] ÷W × 50 (dilution ratio) = 0.92 U/g mass.

2. Take 0.1g of liver, add 1ml reagent one, homogenize in ice bath, centrifuge at 4℃ and 8000g for 10 min, take the supernatant, dilute 500 times, and place it on ice for measurement. Operate according to the determination steps, and calculate ΔA_T= A4-A3 = 0.824-0.543 = 0.281, ΔA_B= A2-A1 = 0.591-0.539 = 0.052

GST (U/g mass) = 0.23 × [(A4-A3) - (A2-A1)] ÷ W × 500 (dilution ratio) = 263.35 U/g mass.

Recent Product Citations：

[1] Wensu Han,Yemeng Yang,Jinglin Gao,et al. Chronic toxicity and biochemical response of Apis cerana cerana (Hymenoptera: Apidae) exposed to acetamiprid and propiconazole alone or combined.

Ecotoxicology. May 2019:28(4):399-411.(IF2.46)

[2] Zhi Zhou,Xiaopeng Yua,Jia Tang,et al. Systemic response of the stony coral Pocillopora damicornis against acute cadmium stress. Aquatic Toxicology. January 2018;(IF3.794)

[3] Le Guan,Muhammad Salman Haider,Nadeem Khan,et al. Transcriptome Sequence Analysis Elaborates a Complex Defensive Mechanism of Grapevine (Vitis vinifera L.) in Response to Salt Stress. International Journal of Molecular Sciences. December 2018;(IF4.183)

[4] Xing Wei,Xuejun Mo,Faliang An,et al. 2′,4′-Dihydroxy-6′-methoxy-3′,5′-dimethylchalcone, a potent Nrf2/ARE pathway inhibitor, reverses drug resistance by decreasing glutathione synthesis and drug efflux in BEL-7402/5-FU cells. Food and Chemical Toxicology. September 2018;(IF3.775)

[5] Qiuli OuYang,Nengguo Tao,Miaoling Zhang. A Damaged Oxidative Phosphorylation Mechanism Is Involved in the Antifungal Activity of Citral against Penicillium digitatum. Frontier in Immunology. February 2018;(IF4.259)

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
BC1150/BC1155	Oxidized Thioredoxin Reductase(TrxR) Activity Assay Kit
BC1210/BC1215	γ-glutamylcysteine Ligase(GCL) Activity Assay Kit
BC1220/BC1225	γ-glutamyl Transpeptidase(γ-GT) Activity Assay Kit