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

Components:

Solution I: Liquid 100 mL×1. Storage at 4℃.

Solution II: Liquid 22 mL×1. Storage at 4℃.

Solution III: Powder×1. Storage at 4℃. Dissolve with 2 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/microplate reader, refrigerated centrifuge, water bath, micro quartz cuvette/96 well UV flat-bottom plate, mortar/homogenizer, transferpettor 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℃ 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/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 25℃ (general species) or 37℃ (mammals) 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℃ (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 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℃ (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

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℃ or 25℃ per minute every milligram protein.

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

2) Calculate by 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℃ or 25℃ per minute every gram tissue sample.

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

3) Calculate by 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℃ or 25℃ per minute every 104 cells.

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

4) Calculate by liquid volume

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

Cpr: 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)

Vrs: Add supernatant liquid volume into the reaction system, 20 μL=0.02 mL;

T: Reaction time, 5 minutes;

W: Sample fresh weight, g;

500: 5 million cells or bacteria;

Vs_1: 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 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)]÷(ε×d)×10^6×Vrv÷(Cpr×Vrs)÷T

=0.38×[(A4-A3)-(A2-A1)] ÷Cpr

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℃ or 25℃ per minute every gram tissue sample.

GST (U/g fresh weight)= [(A4-A3)-(A2-A1)]÷(ε×d)×10^6×Vrv÷Vrs÷Vs_1×W)÷T

= 0.38×[(A4-A3)-(A2-A1)] ÷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℃ or 25℃ per minute every 104 cells.

GST (U/10^4 cell) = [(A4-A3)-(A2-A1)]÷(ε×d)×10^6×Vrv÷(500×Vrs÷Vs_1)÷T

= 0.38×[(A4-A3)-(A2-A1)] ÷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℃ or 25℃ per minute every millilter liquid sample.

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

= 0.38×[(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 mol=1×10^6 μmol;

Vrv: The total volume of the reaction system, 220 μL=2.2×10^{-4} 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 μL=0.02 mL;

T: Reaction time, 5 minutes;

W: Sample fresh weight, g;

Vs_1: 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 25℃ and mammal samples at 37℃.

Experimental Examples：

1. Take 0.1 g of rose and add 1 mL of Reagent I to conduct ice bath homogenization, centrifugation at 4℃ 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:

GST (U/g mass) =0.23 × [(A_4-A_3) - (A_2-A_1)] ÷ W × 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℃ 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:

GST (U/g mass) =0.23 × [(A_4-A_3) - (A_2-A_1)] ÷ W × 50 (dilution multiple) =41.952 U/g mass.

References：

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cerana cerana (Hymenoptera: Apidae) exposed to acetamiprid and propiconazole alone or combined. Ecotoxicology. May 2019:28(4):399-411.(IF2.46)

[2] 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)

[3] 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)

[4] 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)

[5] Chunsheng Li,Xianqing Yang,Ying Xu,et al. Cadmium detoxification induced by salt stress improves cadmium tolerance of multi-stress-tolerant Pichia kudriavzevii. Environmental Pollution. November 2018;(IF5.714)

Related Products：

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BC1180/BC1185	Oxidized Glutathione(GSSG) Content Assay Kit
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