Glutathione S-transferase (GST) Assay Kit

Cat No. GST-M-50
Size: 50 Reactions
Store at 4°C under dark conditions

MOLEQULE-ON®

Components	Quantity
Reagent I	50ml
Reagent II	45ml
Reagent III	Powder (Dissolve with 5 mL of distilled water before use)

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, micropipette, 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 xg 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 xg 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 (Dissolve with 5 mL of distilled water before use) 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.

Calculations

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.

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

2. Calculate by 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.

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

3. Calculate by 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.

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

3. 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.

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

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 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;
Vs1: Volume of Reagent I, 1 mL.

Notes

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 effect 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 ΔAT = A4-A3 = 0.647-0.587 = 0.06, ΔAB= 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 ΔAT= A4-A3 = 0.824-0.543 = 0.281, ΔAB= 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.