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Total Glutathione Peroxidase Activity Assay Kit

Total Glutathione peroxidase (GSH -Px/ GPX) Activity Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation equipment: Spectrophotometer/Microplate Reader

Catalog Number: AK1048-100T-96S

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 SunLong staff in time.

Reagent name
Size
Preservation Condition
Extract solution I
Liquid 120 mL×1
2-8℃
Extract solution II
Liquid 0.6 mL×2
-20℃
Reagent I
Liquid 24 mL×1
2-8℃
Reagent II
Powder×1
2-8℃
Reagent III
Liquid 0.23mL×1
2-8℃
Reagent IV
Powder×1
-20℃
Reagent V
Liquid 0.03 mL×1
2-8℃

Solution Preparation:

1. Extract solution: According to the sample size, Extract solution I: Extract solution II is prepared at the ratio of 3.96mL : 40μL (a total of 4mL, 4T), effective within 3h.
2. Reagent II: Dissolve in 0.163 mL distilled water before use, unused reagent can be stored at 2-8°C for 4 weeks.
3. Reagent IV: Dissolve in 0.531 mL distilled water before use, unused reagent can be stored at -20°C for 4

weeks.

4. Working solution: According to the sample number, Reagent I: Reagent II: Reagent III:Reagent IV is prepared in the ratio of 760μL: 4μL: 8μL: 16μL(a total of 788μL, about 4T), effective within 3h.

5. Reagent V working solution: According to the sample number, reagent I: reagent V is prepared in the ratio of 79μL : 1μL (a total of 80μL, 4T), effective within one day.

Note: Extract solution II, Reagent III and Reagent V are small in volume, and need to use a palm centrifuge to centrifuge the reagent to the bottom before use.

Product Description:

Glutathione peroxidase (GSH-Px/GPX) is an important peroxidative enzyme widely present in the organism, and GPX can be divided into two categories according to the different active centers of the enzyme, one with selenocysteine as the active center, and the other with cysteine as the active center, and the former is present in most of the organisms. GPX can remove hydrogen peroxide, lipid peroxides, etc. produced in the organism, and is an important part of the antioxidant system in the organism. GPX can scavenge hydrogen peroxide and lipid peroxides produced in organisms to reduce the toxic effects of oxidized products on the organism, and is an important part of the antioxidant system in organisms.

Glutathione peroxidase catalyzes the reaction between GSH and peroxides to produce oxidized glutathione (GSSG), and GSSG reacts with NADPH to produce GSH under the action of glutathione reductase (GR). NADPH has a characteristic absorption peak at 340 nm, and the rate of decrease of the absorbance value of the reaction system correlates linearly with the activity of glutathione peroxidase. This kit uses organic peroxides as the substrate and calculates the sum of the two types of GPX activities by measuring the consumption of NADPH per unit time.

Reagents and Equipment Required but Not Provided:

Ultraviolet spectrophotometer/microplate reader, analytical balance, water bath/constant temperature incubator, adjustable pipette, mortar/homogenizer/cell ultrasonic crusher, centrifuge, micro quartz cuvette/96 well UV plate, EP tube, ice and distilled water.

Operation procedure:

I. Sample preparation:(The sample size to be tested can be adjusted appropriately, and the specific proportion can be referred to the literature.)

1. Tissue sample: Add Extract solution according to the ratio of tissue mass (g) : Extract solution volume (mL) = 1:5 ~10 (it is recommended to weigh 0.1g sample and add 1.0 mL Extract solution), after ice bath homogenization, centrifuge at 4℃, 10000g for 10min, take supernatant and placed on the ice for test.

2. Bacteria or cell: The ratio of bacteria/cell amount (106): the volume of Extract solution (mL) is 5~10:1 (it is suggested to take about 5 million bacteria/cells and add 1 mL of Extract solution). Bacteria/cell is split by ultrasonic (placed on ice, 200 W, work time 3 s, interval 6 s, total time 3min). Centrifuge at 4℃ for 10 min at 10000g, take the supernatant and placed on the ice for test.

3. Serum (plasma) sample: Detect sample directly. (If the solution is turbid, centrifuge to take the supernatant and then placed on the ice for test).

II. Determination procedure:

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

2. Preheat the working solution for 10min at 25℃ before use.

3. Operation table(Add the following reagents in 1mL quartz cuvette/96-well UV plate in sequence.)

Reagent (μL)	Test tube (T)	Blank tube (B)
Sample	10	-
Extract solution	-	10
working solution	170	170
Reagent V working solution	20	20

After adding the Reagent V working solution, it is thoroughly mixed for 10s, and the absorption value A1 is immediately determined at 340nm, recorded as A1T and A1B, and incubated at 25℃ for 5 min, the absorption value A2 is determined and recorded as A2T and A2B respectively. ΔAT =A1T -A2T, ΔAB =A1B -A2B, ΔA=ΔAT -ΔAB. The blank tube only need to be measured 1-2 times.

Note: Working solution and reagent V working solution should not be mixed in advance.

III. Calculation:

A. Calculation with 1mL quartz cuvette

1. Calculate by sample protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the consumption of 1 nmol NADPH per minute per milligram of protein at 25°C.

GPX Activity (U/mg prot)= [ΔA÷(ε×d)×109×VT] ÷(Cpr×VS)÷T×F =643.09×ΔA÷Cpr×F

2. Calculate by sample mass

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the consumption of 1 nmol of NADPH in the reaction system per minute every gram tissue at 25°C.

GPX Activity (U/g mass)= [ΔA÷(ε×d)×109×VT] ÷(W×VS÷VE)÷T×F =643.09×ΔA÷W×F

3. Calculate by the number of bacteria or cells

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the consumption of 1 nmol of NADPH in the reaction system per minute every 106 bacteria or cells at 25°C.

GPX Activity (U/106 cell)= [ΔA÷(ε×d)×109×VT] ÷(N×VS÷VE)÷T×F =643.09×ΔA÷N×F

4. Calculate by liquid volume

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the consumption of 1 nmol of NADPH in the reaction system per minute every milliliter liquid at 25°C.

GPX Activity (U/mL)= [ΔA÷(ε×d)×109×VT] ÷VS÷T×F =643.09×ΔA×F ε: The molar extinction coefficient of NADPH, 6.22×103 L/(mol·cm); d: Micro quartz cuvette light path,

1cm; 109: Reduction coefficient, 1mol=109 nmol; V: Reaction system volume, 2×10-4 L; VT: Sample volume,

0.01 mL; VE: Extract solution volume, 1 mL; T: Reaction time, 5 min; W: Sample mass, g; N: The number of cells or bacteria, count by 106; Cpr: Sample protein concentration, mg/mL; F: Dilution multiple.

B. Calculation with 1mL quartz cuvette

Modify d-1cm in the calculation formula to 0.6cm (96 well UV plate light path)

Note:

1. The sample supernatant should be prepared on the day of the experiment and placed on ice. Freeze- thaw will lead to protein denaturation and affect the experimental results.

2. When the enzyme activity of the sample is high, the absorbance value decreases rapidly, resulting in a small ΔA value. The sample can be diluted with the Extract solution and then determined.

3. If the absorbance value decreases slowly, the reaction time after adding the reagent seven working solution can be prolonged appropriately, and the calculation formula can be modified synchronously.

4. The optimal dilution factor of mouse liver is 20-40 times, and the optimal dilution factor of mouse kidney is 4-10 times. Cells and bacteria are not recommended to be diluted.

Experimental examples:

1. Take 0.105g mouse liver and add 1.0 mL Extract solution, after ice bath homogenization. The protein content of the supernatant was 12.38 mg/mL after centrifugation, take the supernatant and diluted 20 times with Extract solution, then follow the determination steps. After determination with 96 well UV plate, calculate ΔAT= A1T-A2T=0.799-0.506=0.293, ΔAB= A1B-A2B= 0.823-0.795=0.028, ΔAT= AT- AB= 0.293-0.028 =0.265. The result is calculated by sample protein concentration:

GPX Activity (U/mg prot)= 1071.81×ΔA÷Cpr×F=458.9 U/mg prot

2. Take 0.101 g mouse kidney and add 1.0 mL Extract solution, after ice bath homogenization. The protein content of the supernatant was 7.83 mg/mL after centrifugation, take the supernatant and diluted 10 times with Extract solution, then follow the determination steps. After determination with 96 well UV plate, calculate ΔAT= A1T-A2T= 0.730-0.532=0.198, ΔAB= A1B-A2B= 0.823-0.795=0.028, ΔAT= AT- AB= 0.198-0.028=0.170. The result is calculated by sample protein concentration:

GPX Activity (U/mg prot)= 1071.81×ΔA÷Cpr×F=232.7 U/mg prot