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Glutathione Peroxidase 4(GPX4)Activity Assay Kit

Glutathione peroxidase 4（GPX4）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: AK1046-100T-48S

Size: 100T/48S

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 70 mL×1	2-8℃
Extract solution II	Liquid 0.6 mL×1	-20℃
Reagent I	Liquid 24 mL×1	2-8℃
Reagent II	Liquid 0.32 mL×1	-20℃
Reagent III	Liquid 0.32 mL×1	2-8℃
Reagent IV	Powder×1	2-8℃
Reagent V	Liquid 0.23 mL×1	2-8℃
Reagent VI	Powder×1	-20℃
Reagent VII	Liquid 30 μL×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 working solution: According to the sample size, Reagent I: Reagent II is prepared in the ratio of 140μL: 20μL (a total of 160μL, 4S), effective within one day.

3. Reagent III working solution: According to the sample size, Reagent I: Reagent III is prepared in the ratio of 140μL: 20μL (a total of 160μL, 4S), effective within one day.

4. Reagent IV: Before use, add 0.163mL distilled water to dissolve, and the unused reagent can be stored at 2-8℃ for 4 weeks .

5. Reagent VI: Before use, add 0.531mL distilled water to dissolve, and the unused reagent can be stored at -20℃ for 4 weeks. Do not freeze and thaw repeatedly.

6. Preparation of working solution: According to the sample size, Reagent I: Reagent IV: Reagent V: Reagent VI is prepared in the ratio of 572μL: 4μL: 8μL: 16μL (a total of 0.6mL, about 4T), effective within 3h.

7. Reagent VII working solution: According to the sample size, Reagent I: Reagent VII is prepared in the ratio of 79μL: 1μL (a total of 80μL, 4T) is prepared according to the sample size, effective within 1d.

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

Product Description:

Glutathione peroxidase 4 (GPX4, EC1.11.1.12) is one of the peroxide- breaking enzymes with selenocysteine as the active center, and is a member of the glutathione peroxidase family. It can catalyze the reaction of glutathione (GSH) with peroxides, reducing peroxides to hydroxyl compounds, thereby protecting cells from free radical damage.

Glutathione peroxidase catalyzes the reaction of GSH with peroxide to produce oxidized glutathione (GSSG), which 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 at which absorbance value of the reaction system decreases is linearly correlated with glutathione peroxidase activity. This kit added GPX4 inhibitor to the reaction system, and calculated GPX4 specific enzyme activity by measuring non-specific enzyme activity and total enzyme activity.

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℃, 12000rpm 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 3 min). 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 ultraviolet spectrophotometer for more than 30 minutes, adjust the wavelength to 340 nm and set zero with distilled water.

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

3. Operation table(Add samples in 96 well UV plate/micro quartz cuvette/ EP tube according to the following steps)

Reagent (μL)

Control tube (C)

Test tube (T)

Sample

10

10

Reagent II working solution

40

-

Reagent III working solution

-

40

Mix well and incubate at 37℃ for 60 min. If you react in an EP tube, transfer the above liquid to a 96-well UV plate/micro quartz cuvette after reaction to continue the experiment

Working solution

130

130

Reagent VII working solution

20

20

After adding the Reagent VII working solution, it was thoroughly mixed for 10s, and the absorption value A1 was immediately determined at 340 nm, recorded as A1C and A1T, and incubated at 25℃ for 5 min , the absorption value A2 was measured and recorded as A2C and A2T respectively. ΔAT =A1T -A2T, ΔAC =A1C -A2C, ΔA=ΔAT -ΔAC. A control tube is required for each test tube.

Note:Working solution and Reagent VII working solution should not be mixed in advance.

III. Calculation:

1. Determine with 96 well UV plate:

(1) Calculate by sample protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the consumption of 1 nmol of NADPH in the reaction system per minute every milligram protein.

GPX4 Activity (U/mg prot)= [ΔA÷(ε×d)×109×VT] ÷(Cpr×VS)÷T×F =1071.81×ΔA÷Cpr×F

(2) Calculate by sample mass

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

GPX4 Activity (U/g mass)= [ΔA÷(ε×d)×109×VT] ÷(W×VS÷VE)÷T×F =1071.81×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 catalyzes the consumption of 1 nmol of NADPH in the reaction system per minute every 106 bacteria or cells.

GPX4 Activity (U/106 cell)= [ΔA÷(ε×d)×109×VT] ÷(N×VS÷VE)÷T×F =1071.81×ΔA÷N×F

(4) Calculate by liquid volume

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

GPX4 Activity (U/mL)= [ΔA÷(ε×d)×109×VT] ÷VS÷T×F =1071.81×ΔA×F ε: The molar extinction coefficient of NADPH, 6.22×103 L/(mol·cm); d: 96 well UV plate light path,

0.6 cm; 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.

2. Determine with micro quartz cuvette:

(1) Calculate by sample protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the consumption of 1 nmol of NADPH in the reaction system per minute every milligram protein.

GPX4 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 catalyzes the consumption of 1 nmol of NADPH in the reaction system per minute every gram tissue.

GPX4 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 catalyzes the consumption of 1 nmol of NADPH in the reaction system per minute every 106 bacteria or cells.

GPX4 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 catalyzes the consumption of 1 nmol of NADPH in the reaction system per minute every milliliter liquid.

GPX4 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.

Note:

1. The sample supernatant should be prepared on the day of the experiment and stored 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 VII 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 0.100 g 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, ΔAC= A1C-A2C= 0.792-0.540= 0.252. ΔA= ΔAT- ΔAC= 0.293-0.252= 0.041, The result is calculated by sample protein concentration:

GPX4 Activity (U/mg prot)= 1071.81×ΔA÷Cpr×F= 70.99 U/mg prot.

2. Take 0.101g 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, ΔAC= A1C-A2C= 0.727-0.541= 0.186, ΔA= ΔAT- ΔAC= 0.198-0.186= 0.012, The result is calculated by sample protein concentration:

GPX4 Activity (U/mg prot)= 1071.81×ΔA÷Cpr×F= 16.43 U/mg prot.

3. Take 10×106 HEK293T and add 0.5 mL Extract solution, after ice bath homogenization. The protein content of the supernatant was 4.191 mg/mL after centrifugation, take the supernatant and then follow the determination steps. The reaction time was extended to 15 min. After determination with 96 well UV plate, calculate ΔAT= A1T-A2T= 0.859-0.251= 0.608, ΔAC= A1C-A2C= 0.841-0.297= 0.544. ΔA= ΔAT- ΔAC= 0.608-0.544= 0.064, The result is calculated by sample protein concentration:

GPX4 Activity (U/mg prot)= [ΔA÷(ε×d)×109×VT] ÷(Cpr×VS)÷T×F= 5.46 U/mg prot.