（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC- K883-M

Specification：48T(24 samples)/96T(48 samples)

Measuring instrument：Microplate reader (340 nm)

Detection range：3.22-44.69U/L

Elabscience® Glutathione Peroxidase 4 (GPX4) Activity Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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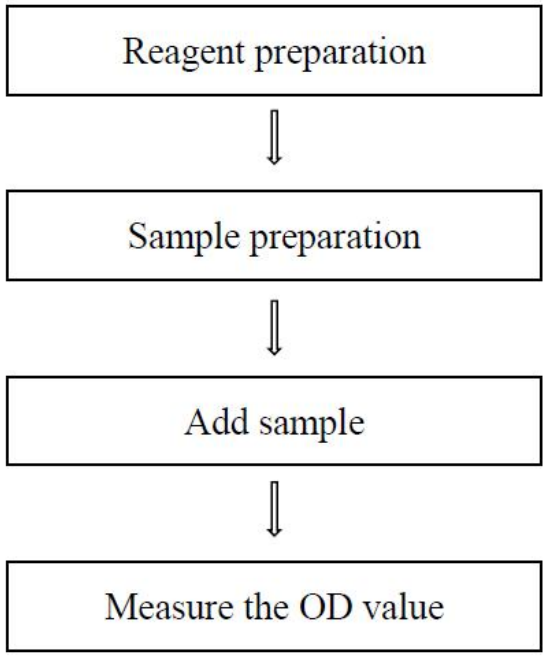
Assay summary



Intended use

This kit can be used to measure GPX4 activity in animal tissue and cell samples.

Detection principle

Glutathione peroxidase 4(GPX4) is a kind of glutathione peroxidase containing selenocysteine, which can reduce phospholipid hydroperoxide to phosphatidyl alcohol, thus protecting the cell membrane from oxidative damage.

GPX4 catalyzes the substrate, and the product consumes the reducing agent with the addition of enzyme reagents.The reducing agent has a maximum absorbance at 340 nm, and the GP4-specific enzyme activity can be calculated by measuring non-specific enzyme activity and total enzyme activity by adding GPX-4 inhibitor to the system.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	25 mL ×1 vial	50 mL × 1 vial	-20℃, 12 months
Reagent 2	Substrate	Powder × 1 vial	Powder × 2 vials	-20℃, 12 months, shading light
Reagent 3	Enzyme Reagent	0.25 mL ×1 vial	0.5 mL × 1 vial	-20℃, 12 months, shading light
Reagent 4	Oxidant	0.15 mL ×1 vial	0.3 mL × 1 vial	-20℃, 12 months, shading light
Reagent 5	Inhibitor	0.25 mL ×1 vial	0.5 mL × 1 vial	-20℃, 12 months, shading light
Reagent 6	Reducing Reagent	Powder × 1 vial	Powder × 2 vials	-20℃, 12 months, shading light
Reagent 7	Accelerant	4 mL ×1 vial	8 mL × 1 vial	-20℃, 12 months, shading light
Reagent 8	Stabilizer	0.5 mL ×1 vial	1 mL × 1 vial	-20℃, 12 months, shading light
	UV Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		

Note: The reagents must be stored strictly according to the preservation conditions

in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (340 nm), Incubator

Reagents:

Double distilled water, Normal saline(0.9% NaCl)

Reagent preparation

① Equilibrate other reagents to room temperature before use.

② Preparation of extract working solution：

For each well, prepare 900 μL of extract working solution (mix well 10 μL of stabilizer and 890 μL of normal saline (0.9% NaCl)). Keep extract working solution on ice during use. The extract working solution should be prepared on spot and used up within 4 h.

③ Preparation of inhibitor working solution：

For each well, prepare 40 μL of inhibitor working solution (mix well 30 μL of buffer solution and 10 μL of inhibitor). Keep inhibitor working solution on ice during use. The inhibitor working solution should be prepared on spot and used up within 1 day.

Other working solution can be prepared during the incubation of the step ③.

④ Preparation of substrate working solution：

Dissolve one vial of substrate with 250 μL of double distilled water, mix well to dissolve. Store at -20°C for 2 days protected from light.

⑤ Preparation of reducing working solution：

Dissolve one vial of reducing reagent with 500 μL of double distilled water, mix well to dissolve. Store at -20°C for 2 days protected from light.

⑥ Preparation of oxidant working solution：

For each well, prepare 40 μL of oxidant working solution (mix well 38 μL of buffer solution and 2 μL of oxidant). Keep oxidant working solution on ice during use. The oxidant working solution should be prepared on spot and used up within 2 h.

⑦ Preparation of reaction working solution：

Before testing, please prepare sufficient reaction working solution according to the test wells. For example, prepare 200 μL of reaction working solution(mix well 108 μL of buffer solution, 2 μL of substrate working solution, 5 μL of enzyme reagent, 5 μL of reducing working solution and 80 μL of accelerant). Keep reaction working solution on ice during use. The reaction working solution should be prepared on spot and used up within 2 h.

Sample preparation

① Sample preparation

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 50 mg tissue in 450 μL extract working solution with a dounce homogenizer at 4°C.

④ Centrifuge at 10000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Cells sample:

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 1×10^6 cells in 200 μL extract working solution with a ultrasonic cell disruptor at 4°C.

④ Centrifuge at 10000×g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection. The supernatant can store at 2-8°C for 1 day.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Note: The protein concentration of the sample should be greater than 0.2 gprot/L, otherwise it will affect the accuracy of results.

② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
10% Mouse liver tissue homogenate	5-50
10% Mouse kidney tissue homogenate	2-6
10% Mouse lung tissue homogenate	2-5
2.05×10^6 293T cells	1
1.9×10^6 4T1 cells	1
2.03×10^6 Jurkat cells	1
2×10^6 Molt-4 cells	1

Note: The diluent is extract working solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① It is recommended to take fresh samples for detection.

② As the sample reaction rate is fast, If A_1 is not determined in time, it may lead to low or no value measured in the sample.

③ After adding oxidant working solution, measure the OD values of each well at 340 nm with microplate reader immediately in 15 s. It's better to measure no more than 10 wells at same time.

④ The protein concentration of the sample should be greater than 0.2 gprot/L, otherwise it will affect the accuracy of results.

Operating steps

① Sample well: Add 20 μL of sample into sample well.
Control well: Add 20 μL of sample into control well.

② Add 40 μL of buffer solution into sample well. Add 40 μL of inhibitor working solution into control well.

③ Mix fully with microplate reader for 5 s, incubate at 37℃ for 30 min

④ Add 140 μL of reaction working solution into each well.

⑤ Add 40 μL of oxidant working solution into each well.

⑥ After adding oxidant working solution, measure the OD values of each well at 340 nm with microplate reader immediately in 15 s recorded as A_1. For tissue sample, incubate at room temperature (25℃) for 5 min, measure the OD values of each well at 340 nm recorded as A_2. For cells sample, incubate at room temperature (25℃) for 15 min, measure the OD values of each well at 340 nm recorded as A_2. As the sample reaction rate is fast, If A_1 is not determined in time, it may lead to low or no value measured in the sample.

Calculation

The sample:

Tissue and cells sample:

Definition: The amount of GPX4 in 1 g protein per minute that catalyze the substrate to produce 1 μmol substance at 25℃ is defined as 1 unit.

$$\text{GPX 4 activity (U/gprot)} = (\Delta A_{sample} - \Delta A_{control}) \div (\varepsilon \times d) \times (V_{total} \div V_{sample}) \div T \times f \div C_{pr}$$

[Note]

ΔA_{sample}: The change OD value of sample well ($\triangle A=A_1-A_2$)

$\Delta A_{control}$: The change OD value of control well ($\triangle A=A_1-A_2$)

ε: the molar extinction coefficient of product at 340 nm, 6.22×10^-3 L/μmol/cm

d: the optical path of cuvette, 0.6 cm.

V_{total}: the total volume of reaction, 0.24 mL.

V_{sample}: the volume of sample, 0.02 mL.

T: the time of tissue sample reaction, 5 min ; the time of cells sample reaction, 15 min.

C_{pr}: The concentration of protein in sample, gprot/L.

f: Dilution factor of sample before test.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three mouse liver samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	6.80	16.20	32.50
%CV	3.1	1.4	1.2

Inter-assay Precision

Three mouse liver samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	6.80	16.20	32.50
%CV	3.4	2.2	0.4

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 98%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	8.5	19	37
Observed Conc. (U/L)	8.5	18.3	36.1
Recovery rate (%)	100	96.3	97.7

Sensitivity

The analytical sensitivity of the assay is 3.22 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Appendix II Example Analysis

Example analysis：

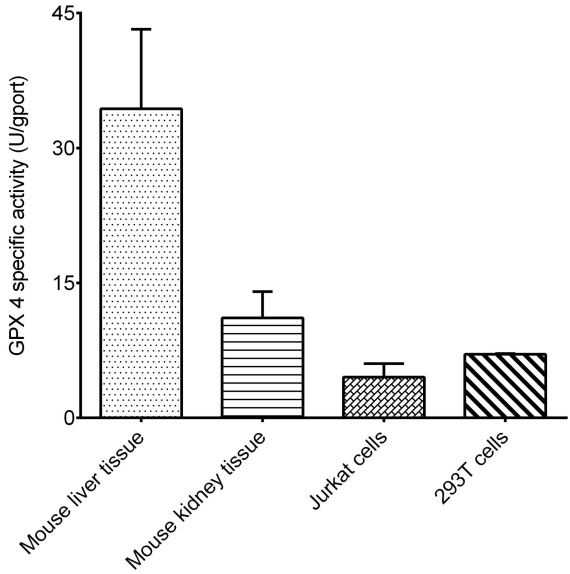
For 10% mouse liver tissue homogenate, dilute for 10 times, take 20 μL of sample and carry the assay according to the operation table. The results are as follows:

The OD value of the sample is 0.143, the OD value of the control is 0.100, the concentration of protein in sample is 6.177 gprot/L and the calculation result is:

GPX 4 activity (U/gprot)

= (0.143-0.100) ÷ (6.22 × 10^-3 × 0.6) × (0.24 ÷ 0.02) ÷ 5 × 10 ÷ 6.177 = 44.76 U/gprot

Detect 10% mouse liver tissue homogenate (the concentration of protein is 6.177 gprot/L), 10% mouse kidney tissue homogenate (the concentration of protein is 5.492 gprot/L), 2.7×10^6 Jurkat cells homogenate (the concentration of protein is 0.639 gprot/L), 2.03×10^6 293T cells homogenate (the concentration of protein is 1.635 gprot/L), according to the protocol, the result is as follows：



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.