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

Catalog No：E-BC- K809-M

Specification：48T(46 samples)/96T(94 samples)

Measuring instrument：Microplate reader（340 nm）

Detection range：3.22-446.95 U/L

Elabscience® Cell Glutathione Peroxidase (GPX) 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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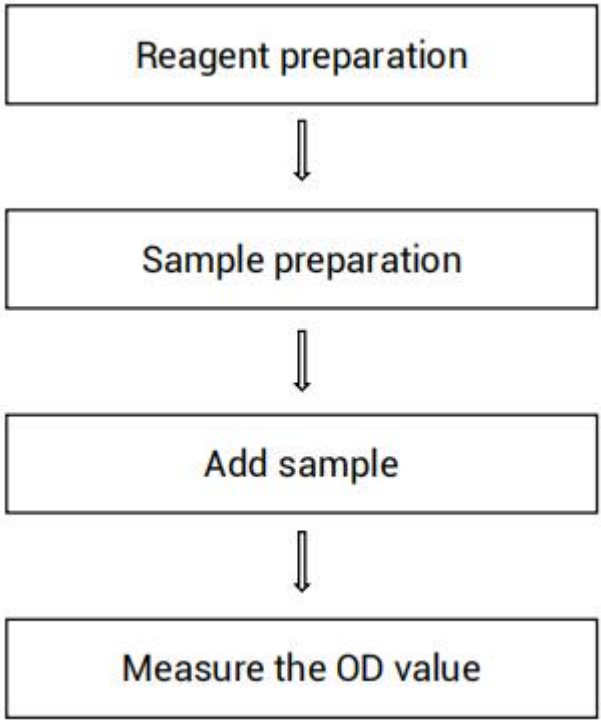
Assay summary



Intended use

This kit can be used to measure glutathione peroxidase (GPX) activity in cell samples.

Detection principle

Glutathione peroxidase (GPX) plays an important role in protecting cells from free radical damage caused by hydrogen peroxide decomposition. The lipid components of cells are prone to react with free radicals, leading to lipid peroxidation. GPX uses glutathione to reduce peroxides to alcohols, thereby preventing cell damage.

GPX catalyzes the substrate, and the product consumes the reducing. The reducing agent has a maximum absorbance at 340 nm. The activity of GPX can be calculated by measuring the change of absorbance value at 340 nm.

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	Reductant	Powder × 1 vial	Powder × 2 vials	-20℃, 12 months, shading light
Reagent 6	Accelerant	4 mL ×1 vial	8 mL ×1 vial	-20℃, 12 months, shading light
Reagent 7	Stabilizer	0.5 mL ×1 vial	1 mL × 1 vial	-20℃, 12 months, shading light
Reagent 8	Cell Lysis Buffer	7 mL ×1 vial	14 mL × 1 vial	-20℃, 12 months
	UV Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

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

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② 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.

③ Preparation of oxidant working solution：
Before testing, please prepare sufficient oxidant working solution according to the test wells. For example, prepare 100 μL of oxidant working solution (mix well 95 μL of buffer solution and 5 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 hours.

④ Preparation of reductant working solution：
Dissolve one vial of reductant with 500 μL of double distilled water, mix well to dissolve. Store at -20°C for 2 days protected from light.

⑤ Preparation of reaction working solution：
Before testing, please prepare sufficient reaction working solution according to the test wells. For example, prepare 1285 μL of reaction working solution (mix well 825 μL of buffer solution, 10 μL of substrate working solution, 25 μL of enzyme reagent, 25 μL of reducing working solution and 400 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 hours.

Sample preparation

① Sample preparation

Cell (adherent or suspension) samples:

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 99 μL cell lysis buffer and 1 μL stabilizer. Crack on ice for 10 minutes, mix well every 5 minutes.

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).

② Dilution of sample

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

Sample type	Dilution factor
1.9×10^6 HL-60 cells	1
1.4×10^6 293T cells	1
2×10^6 CHO cells	1
1.9×10^6 4T1 cells	1
2.03×10^6 Jurkat cells	1

Note: The diluent is cell lysis buffer. For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

If the A_2 of sample well is less than 0.2, the sample must be diluted and test again.

Operating steps

① Sample well: Add 20 μL of sample into sample well.
Blank well: Add 20 μL of cell lysis buffer into blank well.

② Add 180 μL of reaction working solution into each well.

③ Add 40 μL of oxidant working solution into each well.

④ Mix fully with microplate reader for 5 s. After adding oxidant working solution, measure the OD values of each well at 340 nm with microplate reader immediately recorded as A_1. Incubate at room temperature for 10 min, measure the OD values of each well at 340 nm recorded as A_2. As the reaction rate is fast, If A_1 is not determined in time, it may lead to low or no value in the sample.

Calculation

Cell sample:

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

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

[Note]

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

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

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

d: Optical path, 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 reaction, 10 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 HL-60 cell 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/gprot)	98.53	116.90	129.05
%CV	3.10	1.20	1.50

Inter-assay Precision

Three HL-60 cell samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/gprot)	79.97	66.10	58.03
%CV	0.40	3.10	3.40

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 100%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/gprot)	102.29	116.90	131.51
Observed Conc. (U/gprot)	98.53	116.90	129.05
Recovery rate (%)	96.33	100.00	98.13

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 Π Example Analysis

Example analysis：

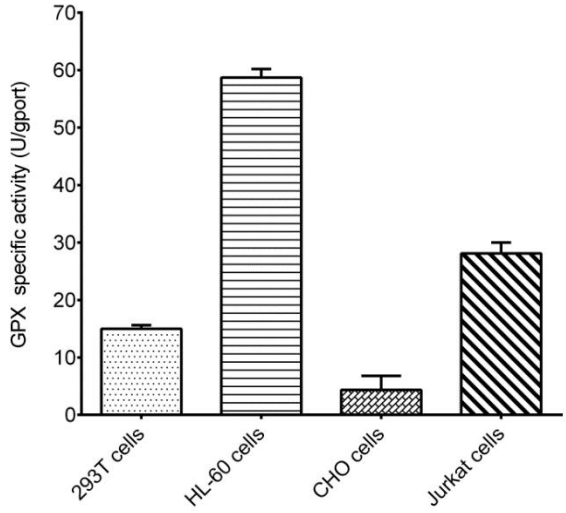
For HL-60 cell, take 20 μL of supernatant and carry the assay according to the operation table. The results are as follows:

The A_1 of the sample is 1.153, the A_2 of the sample is 0.851, $\Delta A_{sample} = A_1 - A_2 = 1.153 - 0.851 = 0.302$. The A_1 of the blank is 1.163, the A_2 of the blank is 1.157, $\Delta A_{blank} = A_1 - A_2 = 1.163 - 1.157 = 0.006$. The concentration of protein in sample is 1.698 gprot/L and the calculation result is:

GPX activity (U/gport)

= (0.302 – 0.006) ÷ (6.22 × 10^-3× 0.6) × (0.24 ÷ 0.02) ÷ 10 ÷ 1.698 = 56.05 U/gport

Detect 293T cell (the concentration of protein is 1.235 gprot/L), HL-60 cell (the concentration of protein is 1.698 gprot/L), CHO cell (the concentration of protein is 1.850 gprot/L) and Jurkat cell (the concentration of protein is 0.68 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.