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CheKine™ Micro Glutathione Peroxidase 4 (GPX4) Activity Assay Kit

Cat #: KTB1642 Size: 48 T/24 S 96 T/48 S

	Micro Glutathione Peroxidase 4 (GPX4) Activity Assay Kit		
REF	**Cat #:** KTB1642	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal Tissues, Cells, Bacteria, Serum, Plasma		
	Storage: Stored at -20℃ for 12 months		

Assay Principle

Glutathione peroxidase 4 (GPX4, EC 1.11.1.12) is the only monomeric selenium-containing antioxidant enzyme in mammals capable of directly reducing membrane-bound lipid peroxides using GSH to protect the lipid bilayer from oxidative damage. GPX4 uses selenocysteine as its active site and is involved in important physiological functions such as cellular membrane antioxidant defense, inhibition of ferroptosis, and sperm maturation. The detection principle of this assay kit is that glutathione peroxidase (GPX) catalyzes the oxidation of GSH by peroxides to produce GSSG, glutathione reductase (GR) catalyzes the reduction of GSSG by NADPH to regenerate GSH, while NADPH is oxidized to $NADP^+$. The GPX activity in the sample can be calculated by measuring the rate of decrease in the absorbance of NADPH at 340 nm. By adding a GPX4 inhibitor to the reaction system, it is possible to measure both total GPX activity and the activity of enzymes other than GPX4; the difference between the two values represents the GPX4 activity.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer Ⅰ	30 mL	60 mL	-20℃
Extraction Buffer Ⅱ	0.3 mL	0.6 mL	-20℃
Reagent Ⅰ	15 mL	30 mL	-20℃
Reagent Ⅱ	0.75 mL	1.5 mL	-20℃
Reagent Ⅲ	0.75 mL	1.5 mL	-20℃
Reagent Ⅳ	50 μL	100 μL	-20℃
Reagent Ⅴ	0.15 mL	0.3 mL	-20℃
Reagent Ⅵ	0.6 mL	1.2 mL	-20℃
Reagent Ⅶ	0.6 mL	1.2 mL	-20℃

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader capable of measuring absorbance at 340 nm
- Incubator
- 96-well UV plate, precision pipettes, disposable pipette tips
- Freezing centrifuge
- Deionized water
- Dounce homogenizer (for tissue samples)

Reagent Preparation

Extraction Buffer Ⅰ: Ready to use as supplied. Equilibrate to room temperature before use. Store at -20℃.

Extraction Buffer Ⅱ: Ready to use as supplied. Equilibrate to room temperature before use. Store at -20℃.

Working Extraction Buffer: Prepare before use, add 10 μL of Extraction Buffer Ⅱ to each 1 mL of Extraction Buffer Ⅰ. Use within the same day after preparation.

Reagent Ⅰ: Ready to use as supplied. Equilibrate to room temperature before use. Store at -20℃.

Reagent Ⅱ: Ready to use as supplied. Equilibrate to room temperature before use. Store at -20℃.

Reagent Ⅲ: Ready to use as supplied. Equilibrate to room temperature before use. Store at -20℃.

Reagent Ⅳ: Ready to use as supplied. Equilibrate to room temperature before use. Store at -20℃.

Working Reagent Ⅴ: Prepare before use, add 0.1 mL of Reagent Ⅴ to each 0.9 mL of Reagent Ⅰ.Valid for 4 hours.

Working Reagent Ⅵ: Prepare before use, add 0.2 mL of Reagent Ⅵ to each 0.8 mL of Reagent Ⅰ.Valid for 4 hours.

Working Reagent Ⅶ: Prepare before use, add 0.2 mL of Reagent Ⅶ to each 0.8 mL of Reagent Ⅰ.Valid for 4 hours.

Sample Preparation

Note: Fresh samples are recommended, as enzyme activity results may be lower when testing frozen samples. All sample preparation and other procedures must be performed on ice, and enzyme activity must be measured on the same day.

1. Animal Tissues: Wash Tissue with cold PBS to remove blood as much as possible. Weigh 0.1 g Tissues, add 0.5 mL Working Extraction Buffer and homogenize on ice. Centrifuge at 12,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested. It is recommended to dilute animal tissue samples 20-fold with Working Extraction Buffer before testing; liver samples should be diluted 40-fold before testing.
2. Cells, Bacteria: Collect 5×10^6 cells or bacteria into the centrifuge tube, add 0.5 mL Working Extraction Buffer to ultrasonically disrupt the cells or bacteria 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 12,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
3. Serum and other liquids: Direct determination. If necessary, can be diluted with Working Extraction Buffer (ensure that the $A_{1\ test}$ is greater than 1.0 and the ΔA_{test} is less than 0.8)

Note: The whole processes need to be carried out on ice, and the enzyme activity should be determined on the same day, to avoid repeated freeze-thaw of the homogenate solution. For additional measurement, it is recommended to use Abbkine Protein Quantification Kit (BCA Assay) (Cat #: KTD3001).

Assay Procedure

1. Preheat the microplate reader for more than 30 min, and adjust the wavelength to 340 nm.
2. Working Regent: Prepare before use; mix thoroughly in the ratio of Reagent Ⅰ: Reagent Ⅱ: Reagent Ⅲ: Reagent Ⅳ= 1600 μL: 150 μL: 150 μL: 10 μL (sufficient for 14 tests).
3. Add the following reagents to the 96-well UV plate:

Reagent	Test Well (μL)	Control Well (μL)
Sample	10	10
Working Reagent Ⅶ	90	0
Working Reagent Ⅵ	0	90
Mix well and incubate at room temperature (25℃) for 30 minutes.		
Working Reagent	130	130
Working Reagent Ⅴ	20	20

Note: Before adding the Working Reagent (after incubating at room temperature for 30 minutes), premix 130 μL Working Reagent and 20 μL Working Reagent V per reaction, and then add 150 μL of the premixed reagent to each reaction well.

4. Mix well. Immediately measure the absorbance value $A_{1\ test}$ and the $A_{1\ control}$ at 340 nm, as well as the $A_{2\ test}$ and the $A_{2\ control}$ after the reaction has proceeded for 5 minutes at 25℃. $\Delta A_{test}=A_{1\ test}-A_{2\ test}$, $\Delta A_{control}=A_{1\ control}-A_{2\ control}$.

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. The reaction temperature has influence on the result. Keep the temperature at 25℃. If $\Delta A_{Test}-\Delta A_{control}$ is less than 0.005, the $A_{2\ test}$ and $A_{2\ control}$ can be extended to 10 minutes, and the value of T in the calculation formula should be adjusted accordingly to 10 or increase the sample quantity appropriately. If ΔA_{Test} is greater than 0.8 or $A_{1\ test}$ is less than 1.0, the sample can be appropriately diluted with Working Extraction Buffer.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

(1) By protein concentration

Active unit definition for GPX4: at 25℃, 1 nmol NADPH oxidation per milligram of protein per minute was catalyzed.

GPX4 activity (U/mg prot)=$[(\Delta A_{test}-\Delta A_{control})\div(\varepsilon\times d)\times V_{Total}\times 10^9]\div(Cpr\times V_{Sample})\div T$**$=1071.8\times(\Delta A_{test}-\Delta A_{control})\div Cpr$**

(2) By sample fresh weight

Active unit definition for GPX4: at 25℃, 1nmol NADPH oxidation per gram of sample per minute was catalyzed.

GPX4 activity (U/g)=$[(\Delta A_{test}-\Delta A_{control})\div(\varepsilon\times d)\times V_{Total}\times 10^9]\div(V_{Sample}\div V_{Sample\ Total}\times W)\div T$**$=535.9\times(\Delta A_{test}-\Delta A_{control})\div W$**

(3) By number of cells or bacteria

Active unit definition for GPX4: at 25℃, 1 nmol NADPH oxidation per 10^6 cells or bacteria of sample per minute was catalyzed.

GPX4 activity ($U/10^6$)=$[(\Delta A_{test}-\Delta A_{control})\div(\varepsilon\times d)\times V_{Total}\times 10^9]\div(N\times V_{Sample}\div V_{Sample\ Total})\div T$**$=535.9\times(\Delta A_{test}-\Delta A_{control})\div N$**

(4) By liquid volume

Active unit definition for GPX4: at 25℃, 1 nmol NADPH oxidation per mL of liquid per minute was catalyzed.

GPX4 activity (U/mL) = $[(\Delta A_{test}-\Delta A_{control})\div(\varepsilon\times d)\times V_{Total}\times 10^9]\div V_{Sample}\div T$**$=1071.8\times(\Delta A_{test}-\Delta A_{control})$**

Where: ε: NADPH molar extinction coefficient 6.22×10^3 L/mol/cm; d: 96-well UV plate light path, 0.75 cm; V_{Total}: Total volume of reaction system, 250 μL=2.5×10^{-4} L; 10^9:1 mol=1×10^9 nmol; Cpr: Protein concentration of supernatant (mg/mL); W: sample mass, g; V_{Sample}: Volume of supernatant added to the reaction system, 10 μL= 1×10^{-2} mL; $V_{Sample\ Total}$: Volume of extraction solution, 0.5 mL; T: Reaction time, 5 min; N: Number of cells or bacteria, expressed in unit of 10^6; for example, for 5×10^6 cells, N is 5.

Recommended Products

Catalog No.	Product Name
KTB1600	CheKine™ Micro Reduced Glutathione (GSH) Assay Kit
KTB1610	CheKine™ Micro Glutathione Oxidized (GSSG) Assay Kit

KTB1620	CheKine™ Micro Glutathione Reductases (GR) Activity Assay Kit
KTB1630	CheKine™ Micro Glutathione S-Transferase (GST) Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.