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CheKine™ Glutathione Peroxidase (GSH-Px) Activity Assay Kit (DTNB Assay)

Cat #: KTB1641 Size: 48 T/48 S 96 T/96 S

	Glutathione Peroxidase (GSH-Px) Activity Assay Kit (DTNB Assay)		
REF	**Cat #:** KTB1641	LOT	**Lot #:** Refer to product label
	Detection range: 6.25-400 nmol/mL		**Sensitivity:** 6.25 nmol/mL
	Applicable sample: Animal and plant tissues, cells, bacteria, fungus, serum (plasma) or other biological fluids.		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Glutathione peroxidase (GSH-Px, EC.1.11.1.9) is an important peroxide decomposition enzyme widely present in the body. GSH-Px can be divided into two categories according to the different enzyme activity centers. One is selenocysteine as the active center and the other is cysteine as the active center. The former exists in most organisms. GSH-Px can remove hydrogen peroxide, lipid peroxides, etc. produced in organisms, reduce the toxic effects of oxidation products on the body, and is an important part of the antioxidant system in organisms. CheKine™ Glutathione Peroxidase (GSH-Px) Activity Assay Kit (DTNB Assay) can detect biological samples such as animal and plant tissues, cells, bacteria fungus, serum, plasma or other biological fluids. In this kit, GSH-Px catalyzes the oxidation of GSH by organic peroxides, producing GSSG. GSH can and DTNB to form compounds with characteristic absorption peaks at 412 nm. The decrease in absorbance at 412 nm can reflect the activity of GSH-Px.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	60 mL	120 mL	4℃
Reagent Ⅰ	Powder×1 vial	Powder×1 vial	4℃, protected from light
Reagent Ⅱ	100 μL	200 μL	4℃, protected from light
Reagent Ⅲ	50 mL	100 mL	4℃
Reagent Ⅳ	15 mL	30 mL	4℃
Reagent Ⅴ	1.5 mL	3 mL	4℃, protected from light
Standard	Powder×1 vial	Powder×1 vial	4℃, protected from light

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 or visible spectrophotometer capable of measuring absorbance at 412 nm
- 96-well microplate or microglass cuvette, precision pipettes, disposable pipette tips,1.5 mL EP tube
- Water bath, freezing centrifuge
- Deionized water, PBS
- Mortar or homogenizer (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Working Reagent I: Prepared before use. Add 5 mL deionized water to Reagent I for 48 T, and 10 mL deionized water to Reagent I for 96 T to fully dissolve. The remaining reagent can also be stored at -20℃ and protected from light for 1 month after aliquoting to avoid repeated freezing and thawing.

Working Reagent II: Prepared before use. Pipette 21.5 μL Reagent II, add 5 mL deionized water and mix thoroughly, and prepared Working Reagent II for use on the same day. Store at 4℃, protected from light.

Reagent III: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent IV: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent V: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Standard: Prepared before use. Add 10 mL deionized water to a bottle, dissolve thoroughly, that is 1 μmol/mL Standard. The remaining 1 μmol/mL Standard can also be stored at -20℃ and protected from light for 1 month after aliquoting to avoid repeated freezing and thawing. Using 1 μmol/mL Standard solution, prepare standard curve dilution as described in the table:

Num.	Standard Volume (μL)	Deionized Water (μL)	Concentration (nmol/mL)
Std.1	200 μL of 1 μmol/mL Standard	300	400
Std.2	200 μL of Std.1 (400 nmol/mL)	200	200
Std.3	200 μL of Std.2 (200 nmol/mL)	200	100
Std.4	200 μL of Std.3 (100 nmol/mL)	200	50
Std.5	200 μL of Std.4 (50 nmol/mL)	200	25
Std.6	200 μL of Std.5 (25 nmol/mL)	200	12.5
Std.7	200 μL of Std.6 (12.5 nmol/mL)	200	6.25
Blank	0	200	0

Note: Always prepare fresh standards per use; Diluted Standard Solution is unstable and must be used within 4 h.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month.

1. Tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 5,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Cells, Bacteria or Fungus: Collect 5×10^6 cells, bacteria or fungus into the EP tube, wash cells, bacteria or fungus with cold PBS, centrifuge at 800 g for 2 min and discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the cells, bacteria or fungus 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 5,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
3. Plasma or other Liquid samples: Test directly.

Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 412 nm, visible spectrophotometer was returned to zero with deionized water.
2. Working Reagent Ⅰ was preheated for 30 min in water bath at 25℃ (non-mammal) or 37℃ (mammal) according to the dosage.
3. Operation table (The following operations are operated in the 1.5 mL EP tube **in turn**):

Reagent	Test Tube (μL)	Control Tube (μL)	Blank Tube (μL)	Standard Tube (μL)
Sample	50	0	0	0
Working Reagent Ⅰ	40	40	0	0
Working Reagent Ⅱ	10	10	0	0
Mix well and incubate accurately at 37℃ for 10 min.			0	0
Reagent Ⅲ	400	400	0	0
Sample	0	50	0	0
Mix well, centrifugation 5,000 g for 10 min at 4℃. Take the supernatant and the following procedures were performed in a 96-well plate or microglass cuvette:			0	0
Supernatant	100	100	0	0
Std. Standard	0	0	0	100
Deionized Water	0	0	100	0
Reagent Ⅳ	100	100	100	100
Reagent Ⅴ	10	10	10	10

4. Mix thoroughly, incubate at 25℃ for 5 min, and measure the absorbance value at 412 nm. The Blank Tube is recorded as A_{Blank}, the Standard Tube is marked as $A_{Standard}$, the Test Tube is marked as A_{Test}, and the Control Tube is marked as $A_{Control}$. Finally calculate $\Delta A_{Test}=A_{Control}-A_{Test}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: The Blank Tube, Standard Tube only need to be done once or twice. Every Test Tube needs to be equipped with a Control Tube. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.01, increase the sample quantity appropriately. If ΔA_{Test} is greater than $\Delta A_{Standard}$ of 400 noml/mL, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

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. Drawing of standard curve

With the concentration of the standard solution as the y-axis and the $\Delta A_{Standard}$ as the x-axis, draw the standard curve and obtain the standard equation. The determination of ΔA_{Test} is substituted into the equation to get y (nmol/mL).

2. Calculation of GSH-Px activity:

(1) Calculated by protein concentration

Active unit definition: Catalyzing the oxidation of 1 nmol GSH per minute per milligram of protein was defined as one unit of enzyme activity.

GSH-Px (U/mg prot)=$y\times V_{Enzyme}\div(V_{Sample}\times Cpr)\div T\times n$**$=y\div Cpr\times n$**

(2) Calculated by sample fresh weight

Active unit definition: Catalyzing the oxidation of 1 nmol GSH per minute per gram of sample was defined as one unit of enzyme activity.

GSH-Px (U/g fresh weight)=y×V_{Enzyme}÷(W×V_{Sample}÷$V_{Total\ sample}$)÷T×n**=y÷W×n**

(3) Calculated by number of cells, bacteria or fungus

Active unit definition: Catalyzing the oxidation of 1 nmol GSH per minute per 10^4 cells, bacteria or fungus was defined as one unit of enzyme activity.

GSH-Px (U/10^4)=y×V_{Enzyme}÷(500×V_{Sample}÷$V_{Total\ sample}$)÷T×n**=0.002y×n**

(4) Calculated by volume of liquid sample

Active unit definition: Catalyzing the oxidation of 1 nmol GSH per minute per milliliter of liquid was defined as one unit of enzyme activity.

GSH-Px (U/mL)=y×V_{Enzyme}÷V_{Sample}÷T×n**=y×n**

V_{Enzyme}: enzyme stimulation reaction volume, 0.5 mL; V_{Sample}: sample volume added, 0.05 mL; $V_{Total\ sample}$: Extraction Buffer volume added, 1 mL; Cpr: sample protein concentration, mg/mL; W: weight of sample, g; 500: The number of cells, bacteria or fungus, 5×10^6, in 10^4; n: sample dilution multiple; T: enzyme stimulation reaction time, 10 min.

Typical Data

The following data are for reference only, y=250.12x-1.1433， R^2=0.9999. And the experimenters need to test the samples according to their own experiments.

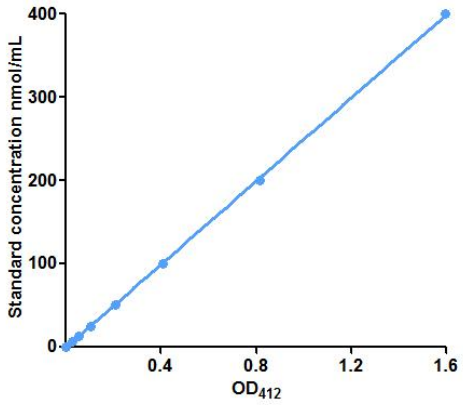

Figure 1. Standard curve.

Example-1: Take 0.1 g of mouse brain, follow the measurement steps, and use a 96-well black plate for detection. Measured $\Delta A_{Test}=A_{Control}-A_{Test}$=0.433-0.304=0.129. Substitute the standard curve and calculate y=31.12. Calculated according to the sample quality, GSH-Px (U/g fresh weight)=y÷W×n=311.2 U/g.

Recommended Products

Catalog No.	Product Name
KTB1320	CheKine™ Micro Plant Soluble Sugar Assay Kit
KTB1330	CheKine™ Micro Blood Glucose Assay Kit
KTB1340	CheKine™ Micro Glycogen 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.