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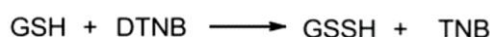
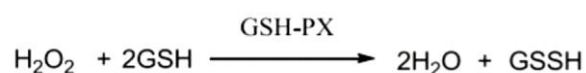
Glutathione Peroxidase (GPx) Assay Kit

BC4403-01 (50Tests/24Samples)

FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS

Product Description

Glutathione Peroxidase (GSH-Px) can promote the reaction of hydrogen peroxide (H₂O₂) and reduced glutathione to produce H₂O and oxidized glutathione (GSSG). The activity of glutathione peroxidase can be expressed by the rate of enzymatic reaction. The activity of glutathione can be calculated by measuring the consumption of reduced glutathione. Hydrogen peroxide (H₂O₂) and reduced glutathione can react without catalysis of GSH-Px, so the portion of GSH reduction by non-enzymatic reaction should be subtracted. GSH can react with dinitrobenzoic acid to produce 5-thio-dinitrobenzoic acid anion, which showed a stable yellow colour. Measure the absorbance at 412nm, and calculate the amount of GSH.



Kit components

Reagent	Volume	Storage
Extract solution	50mL	4°C
Reagent I	5mL	4°C
Reagent II	Powder	4°C
Reagent III	20μL	4°C
Reagent IV	22mL	4°C
If crystals are found in the bottom of the bottle, warm in a 50°C water bath till it dissolves. If crystals persists after heating, the supernatant can be used for the assay.		
Reagent V	15mL	4°C
Reagent VI	Powder	4°C
Standard (10mg reduced glutathione)	Powder	4°C
Add 0.405mL distilled water to get a standard concentration of 80μmol/mL.		
Diluent	35mL	4°C

Reagent Preparation

Reagent II

Stock: Dissolve Reagent II in 0.405mL distilled water. This solution can be stored at –20°C for 4 months.

Working solution: Take 5μL Reagent II Stock and mix with 4995mL Diluent. Always prepare fresh before the assay.

Reagent III

Working solution: Dilute 3μL Reagent III with 10mL distilled water. Prepare according to the number of samples to be assayed. Always prepare fresh before the assay. Do not store the working solution.

Reagent VI

Working solution: Dissolve entire contents of Reagent VI in 25mL of Diluent. Mix well until fully dissolved. Working solution is stable for 2 weeks at 2–8°C and for 2 months at –20°C. Prepare aliquots before freezing to avoid multiple freeze-thaw cycles.

Standard

Stock: Add 0.405mL distilled water to get a standard concentration of 80μmol/mL.

Working Standard: Dilute the Standard solution (80μmol/mL) to 0.08μmol/mL with Diluent.

Note:

- Avoid repeated freeze-thaw cycles by storing in aliquots.

Reagents and Equipment Required but Not Provided

Constant temperature water bath, cooling centrifuge, spectrophotometer / microplate reader, micro glass cuvette / 96-well flat bottom plates and distilled water.

Protocol

I. Sample preparation

Tissue: Add 1mL Extract Solution to 0.1g tissue. Homogenate in ice and centrifuge at 5000rpm at 4°C for 10 minutes. Take the supernatant and place it on ice for assay. (If the supernatant is not clear, centrifuge for 3 minutes more).

Bacteria or cells: Add 1mL Extract Solution to 5 million cells. Subject to ultrasonication while keeping the samples in an ice bath (power 300W, sonication 3seconds, interval 7seconds for 3 minutes). Centrifuge at 5000 rpm at 4°C for 10 minutes. Take the supernatant and place it on ice for assay. (If the supernatant is not clear, centrifuge for 3 minutes more).

Serum: Use directly.

II. Assay procedure

- Preheat the spectrophotometer/microplate reader for 30 minutes, adjust wavelength to 412 nm and set zero with distilled water.
- Perform the subsequent assay in a 1.5mL micro centrifuge tube. Add the reagents in the order as mentioned in the table below.

Reagent	Test tube (T)	Control tube(C)
Sample (supernatant/serum)	20μL	-
Reagent I	20μL	20μL
Incubate for 5 minutes at 37°C		
Reagent II working solution	20μL	20μL
Reagent III working solution	10μL	10μL
Incubate for 15 minutes at 37°C		
Reagent IV	200μL	200μL
Sample (supernatant/serum)	-	20μL

- Centrifuge at 4000rpm at room temperature for 5 minutes and transfer the supernatant into a new 1.5mL micro centrifuge tube or 96 well plate.
- Continue the assay as given in the table below

Reagent	Test tube (T)	Control tube (C)	Standard tube (S)	Black tube (B)
Diluent	-	-	-	100μL
Supernatant	100μL	100μL	-	-
Working standard	-	-	100μL	-
Reagent V	100μL	100μL	100μL	100μL
Reagent VI	25μL	25μL	25μL	25μL

- Well mix. then placed at room temperature for 15 minutes, the absorbance at 412 nm is measured. The absorbance is recorded as A_T , A_C , A_S and A_B , respectively

$$\Delta A_T = A_C - A_T$$

$$\Delta A_S = A_S - A_B.$$

Calculation

1. Calculation of inhibition percentage:

$$\text{Inhibitory percentage} = (A_C - A_T) / (A_S - A_B) \times 100\%$$

It is ideal to keep the inhibition percentage of the sample is within the range of 30-70%, and the closer it is to 50%, the more accurate it is. If inhibition percentage is less than 30% or more than 70%, it is usually necessary to adjust sample volume or sample preparation. If inhibition percentage is high, the sample should be diluted properly. If inhibition percentage is low, the sample with high concentration should be prepared again.

2. Calculation of GPX activity:

a) Protein concentration

Unit definition: One unit of enzyme is defined as the amount of enzyme which catalyzes the oxidation of 1nmol of GSH in reaction system per minute for every milligram of protein.

$$\begin{aligned}\text{GPX (U/mg protein)} &= \Delta A_T \div (\Delta A_S \div C_S) \times 1000 \times V_{EV} \div (C_{pr} \times V_{SV}) \div T \\ &= 200 \times \Delta A_T \div \Delta A_S \div C_{pr}\end{aligned}$$

b) Sample weight:

Unit definition: One unit of enzyme is defined as the amount of enzyme which catalyzes the oxidation of 1nmol of GSH in reaction system per minute for every gram of sample.

$$\begin{aligned}\text{GPX (U/g weight)} &= \Delta A_T \div (\Delta A_S \div C_S) \times 1000 \times V_{EV} \div (V_{SV} \div V_{TV} \times W) \div T \\ &= 200 \times \Delta A_T \div \Delta A_S \div W\end{aligned}$$

c) Cells

Unit definition: One unit of enzyme is defined as the amount of enzyme which catalyzes the oxidation of 1nmol of GSH in reaction system per minute for every 10^4 cells.

$$\begin{aligned}\text{GPX (U/}10^4\text{ cells)} &= \Delta A_T \div (\Delta A_S \div C_S) \times 1000 \times V_{EV} \div (N \times V_{SV} \div V_{TV}) \div T \\ &= 200 \times \Delta A_T \div \Delta A_S \div N\end{aligned}$$

d) Liquids (Serum/Plasma)

Unit definition: One unit of enzyme is defined as the amount of enzyme which catalyzes the oxidation of 1nmol of GSH in reaction system per minute for every millilitre of liquid.

$$\begin{aligned}\text{GPX (U/mL)} &= \Delta A_T \div (\Delta A_S \div C_S) \times 1000 \times V_{EV} \div V_{SV} \div T \\ &= 200 \times \Delta A_T \div \Delta A_S\end{aligned}$$

C_S : Concentration of standard mixtures, 0.08 $\mu\text{mol/mL}$

V_{EV} : Volume of enzymatic reaction system, 0.25mL

V_{SV} : Sample volume contained in sample mixtures, 0.02mL

V_{TV} : Extraction solution volume, 1mL

C_{pr} : Supernatant protein concentration, mg/mL

T : Reaction time, 5 minutes

N : The amount of cells

W : Sample weight, g

1000 : 1 μmol = 1000 nmol

Note

- If initial absorbance is greater than 1.5, dilute the sample with Extract Solution and repeat the assay.
- It is recommended not to perform assay with too many samples, as increase in incubation time will result in inaccurate results.