Glutathione Peroxidase (GSH-Px) Activity Assay Kit

Catalog Number EEA010 (96 tests)

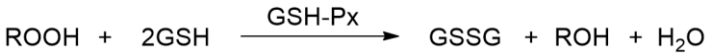
Rev 1.0

For safety and biohazard guidelines, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

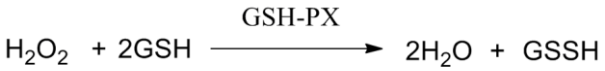
Product description

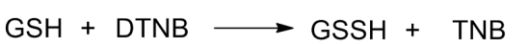
This kit can be used to measure Glutathione peroxidase (GSH-Px) activity of serum, plasma, tissue, cells, and cell culture supernatant samples.

Glutathione peroxidase (GSH-Px) is an important enzyme that catalyzes decomposition of hydrogen peroxide. GSH specifically catalyzes the reaction between GSH and hydrogen peroxide, protecting cell membrane structure and keeping membrane function integrity. Se-cysteine is the active center of the GSH-Px. Determination of GSH-Px activity in organism can be an indicator of selenium level as Se is essential section of GSH-Px.



Glutathione peroxidase (GSH-Px) can promote the reaction of hydrogen peroxide (H_2O_2) and reduced glutathione to produce H_2O 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 (H_2O_2) 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 presents with a stable yellow color. GSH can be calculated by measuring the absorbance of the anions in solution at 412 nm.





Contents and storage

Kit and components are shipped at 2-8 ℃. An unopened kit can be stored at 2-8 ℃ for 12 months.

Components	Quantity (96 tests)
Stock Solution	0.5 mL
Acid Reagent	50 mL
Phosphate	12 mL
DTNB Solution	7 mL
GSH Standard	3.07 mg
GSH Standard Stock Diluent	1.5ml ×2 vials
Microplate	1 plate
Plate Sealer	2 pieces

Required materials

- Distilled or deionized water
- Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)
- Microtiter plate reader with software capable of measurement at or near 412 nm
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solution
- Incubator capable of maintaining 37 ℃.

Procedural guidelines

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

Sample preparation guidelines

Sample requirements

- The samples should not contain SDS, Tween-20, NP-40, Triton X-100 and other detergents, and should not contain DTT, 2-merhydryl ethanol, or other reducing reagents.

Serum and plasma samples: Dilute the sample for testing as required. If the sample is turbid, centrifuge at 10000 g for 10 min at 4 ℃, then take the supernatant for detection.

Cell culture supernatant sample: Detect directly. If there is turbidity, centrifuge at 3100 g for 10 min. Take the supernatant to preserve it on ice for detection.

Tissue sample:

- Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8 ℃.
- Absorb the water with filter paper and weigh.
- Homogenize at the ratio of the volume of PBS (0.01 M, pH 7.4) (2-8 ℃) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4 ℃.
- Take the supernatant to preserve it on ice for detection.

Meanwhile, determine the protein concentration of supernatant (Catalog No. 23227).

If not detected on the same day, the tissue sample (without homogenization) can be stored at -80 ℃ for 1 month.

Cells:

- Collect the cells (For adherent cells, the cell scraper rather than trypsin is recommended) and wash the cells with PBS (0.01 M, pH 7.4) for 1~2 times.
- Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment.
- Add homogenization medium at a ratio of cell number (10^6): PBS (0.01 M, pH 7.4) (μL) =1: 300-500. Sonicate or grind with hand-operated in ice water bath.
- Centrifuge at 1500 g for 10 min, then take the supernatant and preserve it on ice for detection.

Meanwhile, determine the protein concentration of supernatant (Catalog No. 23227).

If not detected on the same day, the cells sample (without homogenization) can be stored at -80 ℃ for 1 month.

Prepare samples

1. The optimal sampling volume is different for different species, and it is recommended to take 2~3 samples to do a pre-experiment to determine the optimal sampling volume before formal experiment.
2. The Inhibition ratio that can be detected by this kit is 10-50%, where the optimal inhibition ratio is 25-45%. When the inhibition ratio is 25-45%, the corresponding sampling volume is the optimal sampling volume.

$$\text{Inhibition ratio} = \frac{\text{OD}_{\text{Non-enzyme}} - \text{OD}_{\text{Enzyme}}}{\text{OD}_{\text{Non-enzyme}}} \times 100\%$$

3. If inhibition ratio > 50%, dilute the sample or decrease the sampling volume, then take the test. If inhibition ratio < 10%, increase the concentration of sample or increase the sampling volume.
4. The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
Human serum	1
Mouse serum	3-5
Rat serum	5-8
10% Mouse brain tissue homogenate	1
10% Rat liver tissue homogenate	30-60
HepG2 cells (5 mgprot/mL)	1
10% Epipremnum aureum leaves tissue homogenate	1
10% Chinese cabbage leaves tissue homogenate	3-5

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4)

Stock Solution application solution

Dilute the stock solution with distilled water 1:100. Prepare the fresh solution before use. The prepared solution can be stored at 2-8 ℃ for 3 days.

GSH Standard Stock Diluent application solution

Dilute 1 part of the GSH standard stock with 9 part of distilled water. Prepare the fresh solution before use. If the GSH standard stock diluent is frozen, thaw at 65 ℃. The prepared solution can be stored at 2-8 ℃ for 7 days.

1 mmol/L GSH standard solution

Dissolve a vial of GSH standard with GSH standard stock diluent application solution to a final volume of 10 mL before use and mix fully. Prepare fresh solution before use. The unused solution can be aliquoted into smaller quantities and stored at -20 ℃ for 1 month..

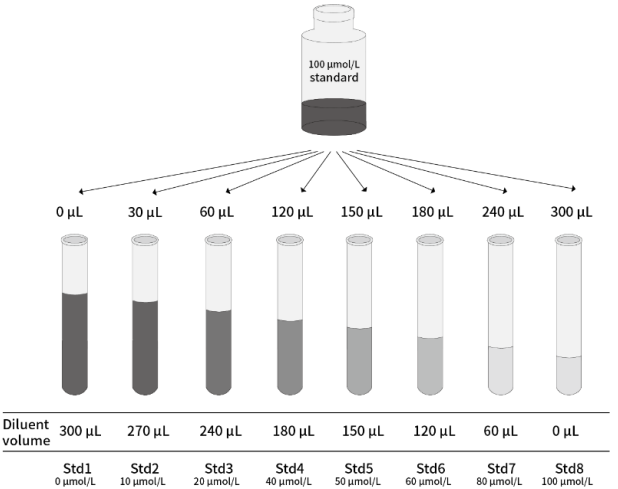
100 μmol/L GSH standard solution

Dilute 1 part of 1 mmol/L GSH standard solution with 9 part of GSH standard stock diluent application solution and mix fully. Prepare fresh solution before use. The prepared solution can be stored at 2-8 ℃ for 7 days.

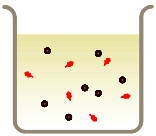
Prepare diluted standards

Note: Use glass or plastic tubes for diluting standards.

Dilute 100 μmol/L GSH standard solution with GSH standard stock diluent application solution to a serial concentration. The recommended dilution gradient is as follows: 0, 10, 20, 40, 50, 60, 80, 100 μmol/L.



Assay Protocol



Enzymatic reaction

a. **Non-enzyme tube**: take 20 μL of 1 mmol/L GSH standard into a 1.5 mL microcentrifuge tube.

   Enzyme tube: take 20 μL of 1 mmol/L GSH standard, 20 μL of sample into 1.5 mL microcentrifuge tube and mix fully.

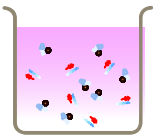
b. Preheat the tubes at 37 ℃ water bath for 5 min. Preheat stock solution application solution at 37 ℃ for 5 min at the same time.

c. Add 10 μL of stock solution application solution to the tubes and mix fully. React at 37 ℃ for 5 min.

d. **Non-enzyme tube:** add 200 μL of acid reagent and 20 μL of sample to the tubes.

   Enzyme tube: add 200 μL of acid reagent to the tubes.

e. Mix fully with a vortex mixer and centrifuge at 3100 g for 10 min, and take 100 μL of the supernatant for chromogenic reaction.



Chromogenic reaction

a. **Non-enzyme wells:** Take 100 μL of supernatant of non-enzyme tubes to the wells.

   Enzyme wells: Take 100 μL of supernatant of enzyme tubes to the wells.

   Standard wells: Take 100 μL of GSH standard solution with different concentrations to the wells.

b. Add 100 μL of phosphate to each well.

c. Add 50 μL of DTNB solution to each well.

d. Oscillate for 10 s with microplate reader and stand for 5 min. Measure the OD values at 412 nm with microplate reader.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.
2. Subtract the mean OD value of the blank (Std1) from all standard readings. This is the absoluted OD value.
3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or EXCEL).

Serum (plasma) and other liquid sample:

Unit definition: The amount of GSH-PX in 0.1 mL of sample that catalyzes the consumption of 1 μmol/L GSH with deducting the effect of the non-enzyme reaction at 37 ℃ for 5 minutes is defined as 1 unit.

$$\text{GSH-Px activity (U)} = (\Delta A_{412} - b) \div a \times \frac{0.23 + V}{0.03 + V} \times \frac{0.1^*}{V} \times f$$

Tissue and cells sample:

Unit definition: The amount of GSH-PX in 1 mg of protein that catalyzes the consumption of 1 μmol/L GSH with deducting the effect of the non-enzyme reaction at 37℃ for 5 minute is defined as 1 unit.

$$\text{GSH-Px activity (U/mgprot)} = (\Delta A_{412} - b) \div a \times \frac{0.23 + V}{0.03 + V} \div (V \times C_{pr}) \times f$$

[Note]

y: The absolute OD value of standard;

x: The concentration of standard;

a: The slope of standard curve;

b: The intercept of standard curve.

ΔA_{412}: The absolute OD value of sample ($OD_{Non\text{-}enzyme\ tube}$-$OD_{Enzyme\ tube}$).

(0.23+V)/(0.03+V): Dilution factor of sample in enzymatic reaction.

0.1*: The volume of sample in definition.

V: The volume of sample added to the reaction system.

f: Dilution factor of sample before test.

C_{pr}: Concentration of protein in sample (mgprot/L)

To easy calculate the test results, refer to the calculation file available on the webpage.

Example analysis

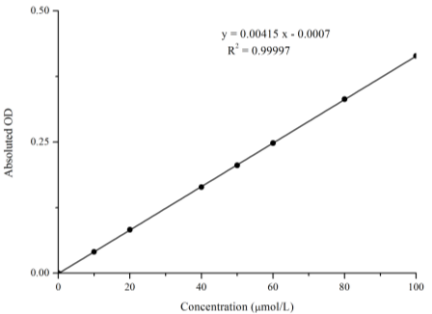
Dilute mouse serum with normal saline (0.9% NaCl) for 4 times, take 20 μL of diluted sample and carry the assay according to the operation table. The results are as follows: standard curve: y = 0.00415x - 0.0007, the average OD value of the non-enzyme well is 0.381, the average OD value of the enzyme well is 0.263, and the calculation result is:

$$\text{GSH-Px activity (U)} = (0.381 - 0.263 + 0.0007) \div 0.00415 \times 5 \times 5 \times 4 = 2860.24 \text{ U}$$

Performance characteristics

- Standard curve (example)

The following data were obtained for the various standards over the range of 0–100 μmol/L standard.

Concentration (μmol/L)	OD at 412 nm	Standard Curve
100.00	0.482	
80.00	0.400	
60.00	0.316	
50.00	0.274	
40.00	0.232	
20.00	0.151	
10.00	0.109	
0.00	0.068	

- Inter-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U)	1700.00	2700.00	3125.00
%CV	5.9	7.5	5.2

CV = Coefficient of Variation

- Intra-assay Precision

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U)	127.00	190.00	220.00
%CV	1.0	3.0	3.0

CV = Coefficient of Variation

- Expected values

This assay was tested with mouse serum samples at dilutions from 1:4 in normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4).

Sample Type	Range (**U**)	Average (**U**)
Mouse serum	114.96-140.32	127.64

- 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 99%.

	Sample 1 (low conc.)	Sample 2 (middle conc.)	Sample 3 (high conc.)
Expected Conc. (U)	1700.00	2700.00	3125.00
Observed Conc. (U)	1700	2754	2968.75
Recovery rate (%)	100	102	95

- Recommended Plate Set Up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S1'	S9	S9'	S17	S17'	S25	S25'	S33	S33'
B	B	B	S2	S2'	S10	S10'	S18	S18'	S26	S26'	S34	S34'
C	C	C	S3	S3'	S11	S11'	S19	S19'	S27	S27'	S35	S35'
D	D	D	S4	S4'	S12	S12'	S20	S20'	S28	S28'	S36	S36'
E	E	E	S5	S5'	S13	S13'	S21	S21'	S29	S29'	S37	S37'
F	F	F	S6	S6'	S14	S14'	S22	S22'	S30	S30'	S38	S38'
G	G	G	S7	S7'	S15	S15'	S23	S23'	S31	S31'	S39	S39'
H	H	H	S8	S8'	S16	S16'	S24	S24'	S32	S32'	S40	S40'

[Note]: A-H, standard wells; S1-S40, Non-enzyme wells; S1'-S40', Enzyme wells.

- Sensitivity

The analytical sensitivity of the assay is 34.3 U. 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.

Limited product warranty

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