Reduced Glutathione (GSH) Colorimetric Assay Kit

Catalog No: E-BC-K030-M

Method: Colorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Instrument: Microplate reader

Sensitivity: 2 μmol/L

Detection range: 2-100 μmol/L

- Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

This kit can measure GSH content in serum, plasma, cells, cell culture supernatant and tissue samples.

▲Background

Reduced Glutathione (GSH) is a kind of low molecular scavenger, which can remove O_2^-, H_2O_2, LOOH. GSH is a small molecule peptide which composed of glutamic acid, glycine and cysteine, and it is the main thiol compound of non-protein in the organization. GSH is the substrate of GSH-Px and GSH-ST which is indispensable for decomposing hydrogen peroxide of these two enzymes, and it can stabilize the enzyme containing thiol and prevent hemoglobin and other auxiliary factors from the oxidative damage. Recently, it is proved that GSH is also involved in the recovery of VE to the reduction state. When lacking or depletion of GSH, it may cause producing toxic effects or increasing the toxic effects of many chemicals or environmental factors. It may be related to the increase of oxidative damage, so the amount of GSH is a vital factor to measure the body's antioxidant ability. GSH plays an important role in the researches process of prevention, recovery and treatment of atherosclerosis, coronary heart disease, anti-aging, anti-tumor, prevention, prevention of Alzheimer's disease and other diseases.

▲Detection principle

Reduced GSH can react with Dinitrobenzoic acid (DNTB) to form a yellow complex which can be detected by colorimetric assay at 405 nm and calculate the reduced GSH content indirectly.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Acid Reagent	12 mL × 1 vial	2-8℃ ,6 months, shading light
Reagent 2	Phosphate	12 mL × 1 vial	2-8℃ ,6 months
Reagent 3	DTNB Solution	1.5 mL × 2 vials	2-8℃ ,6 months, shading light
Reagent 4	GSH Standard	3.07 mg × 2 vials	2-8℃ ,6 months
Reagent 5	GSH Standard Stock Diluent	1.5 mL× 2 vials	2-8℃ ,6 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

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.

▲Materials prepared by users

Instruments

Microplate reader(405-414 nm), Micropipettor, Vortex mixer

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL, 5 mL)

Reagents

Double distilled water, Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4)

▲Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

▲Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲The key points of the assay

1. The supernatant after centrifugation must be clarified.
2. Prevent the formulation of bubbles when the supernatant is transferred into the microplate.

Pre-assay preparation

▲Reagent preparation

1. Preparation of GSH standard diluent:
   Dilute the GSH standard stock diluent with double distilled water at a ratio of 1:9. Prepare the fresh solution before use.
2. Preparation of 1 mmol/L GSH standard solution:
   Dissolve 3.07 mg of GSH standard with 10 mL of GSH standard diluent and mix fully. Prepare the fresh solution before use.

▲Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

Sample requirements

DTT, 2-mercaptoethanol and other reductive substances should not be added in the samples.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (2-100 μmol/L).

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

Sample type	Dilution factor
Human serum	1
Human plasma	1
10% Mouse brain tissue homogenization	1
10% Mouse liver tissue homogenization	1
Hela cell homogenization（0.999 mgprot/mL）	1
Rat serum	1
Rat plasma	1
Mouse serum	1
10% Carrot tissue homogenization	1
293T supernatant	1

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

Assay protocol	
Ambient temperature	25-30℃
Optimum detection wavelength	405 nm

Instructions for the use of transferpettor:

(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.

(2) Don't add the liquid outside the tips into the reaction system when pipetting each reagent.

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A-H, standard wells; S1, blank wells; S2-S80, sample wells.

▲Operating steps

1. The preparation of standard curve

Dilute 1 mmol/L GSH standard solution with GSH standard diluent to a serial concentration. The recommended dilution gradient is as follows: 0, 10, 20, 40, 50, 60, 80, 100 μmol/L.

2. The measurement of samples

(1) Preparation of sample supernatant: take 0.1 mL of sample, add 0.1 mL of reagent 1 and mix fully. Centrifuge at 4500 g for 10 min. Take the supernatant for detection.

(2) Add 25 μL reagent 3 to each tube.

(3) Blank well: Add 100 μL of reagent 1.

Standard well: Add 100 μL of standard solution with different concentration.

Sample well: Add 100 μL of supernatant.

(4) Add 100 μL of reagent 2 to each tube.

(5) Mix fully for 1 min and stand for 5 min at room temperature. Measure the OD values of each well at 405 nm with microplate reader.

▲Operation table

	Blank well	Standard well	Sample well
Reagent 3 (μL)	25	25	25
Reagent 1 (μL)	100		
GSH standard solution with different concentration (μL)		100	
Supernatant (μL)			100
Reagent 2 (μL)	100	100	100
Mix fully for 1 min and stand for 5 min at room temperature. Measure the OD values of each well at 405 nm with microplate reader.			

▲Calculation

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. If the sample have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. The actual concentration is the calculated concentration multiplied by dilution factor.

The standard curve is: y= ax + b.

1. Serum (plasma) and other liquid sample:

$$\text{GSH content (μmol/L)} = (\Delta A_{405}\text{-b}) \div a \times 2^{*} \times f$$

2. Tissue and cells sample:

$$\text{GSH content (μmol/gprot)} = (\Delta A_{405}\text{-b}) \div a \times 2^{*} \times f \div C_{pr}$$

Note:

y: $OD_{Standard}$ – OD_{Blank} (OD_{Blank} is the OD value when the standard concentration is 0)

x: The concentration of standard.

a: The slope of standard curve.

b: The intercept of standard curve.

ΔA_{405}: OD_{Sample} - $OD_{Contrast}$

2*: Dilution factor of in the preparation step of sample supernatant, 2 times.

f: Dilution factor of sample before test.

C_{pr}: Concentration of protein in sample, gprot/L

Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 6 months.
4. Do not use components from different batches of kit.
5. If the GSH content is calculated by protein concentration, the protein concentration of the sample needs to be determined separately (E-BC-K318-M).

Appendix I Performance characteristics

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Detection range	2-100 μmol/L	Average intra-assay CV (%)	1.9
Sensitivity	2 μmol/L	Average inter-assay CV (%)	3.2
Average recovery rate (%)	96		

▲Example analysis

Take 0.1 mL of human serum sample, add 0.1 mL of reagent 1, mix fully and centrifuge at 4500 g for 10 min, then take prepared supernatant, carry the assay according to the operation table. The results are as follows:

standard curve: y = 0.00383x – 0.00251, the average OD value of the sample is 0.080, the average OD value of the blank is 0.047, and the calculation result is:

$$\text{GSH content (μmol/L)} = (0.080 - 0.047 + 0.00251) \div 0.00383 \times 2$$
$$= 18.54 \text{ μmol/L}$$

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲ Serum

Collect fresh blood and stand at 25℃ for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4℃ . Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection. If not detected on the same day, the serum can be stored at-80℃ for a month.

▲ Plasma

Take fresh blood into the tube which has anticoagulant, centrifuge at 1000-2000 g for 10 min at 4℃ . Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice for detection. If not detected on the same day, the plasma can be stored at-80℃ for a month.

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

▲ Cells

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

Note:

1. Homogenized medium: 50 mM Tris-HCl (pH 7.4) including 150 mM NaCl, 1% NP-40, 1 mM EDTA.
2. Homogenized method:
   (1) Hand-operated: Weigh the tissue and mince to small pieces (1 mm^3), then put the tissues pieces to glass homogenized tube. Add homogenized medium into homogenized tube, place the tube into the ice bath with left hand, and insert the glass tamping rod vertically into the homogenized tube with the right hand to grind up and down for 6-8 min.

   Or put the tissue into the mortar, and add liquid nitrogen to grind fully. Then add the homogenized medium to homogenize.

   (2) Mechanical homogenate: Weigh the tissue to EP tube, add the homogenized medium to homogenize the tissue with homogenizer instrument (60 Hz, 90s) in the ice bath. (For samples of skin, muscle and plant tissue, the time of homogenization can be properly prolonged.)

   (3) Ultrasonication: Treat the cells with ultrasonic cell disruptor (200 W, 2 s/time, interval for 3 s, the total time is 10 min).

▲ Notes for sample

1. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
2. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.
3. If a lysis buffer is used to prepare tissue or cell homogenates, there is a possibility of causing a deviation due to the introduced chemical substance.