PRODUCT INFORMATION & MANUAL

Reduced Glutathione/GSH Assay Kit (Colorimetric)

NBP3-25795

For research use only.
Not for diagnostic or therapeutic procedures.

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Reduced Glutathione/GSH Assay Kit (Colorimetric)

Catalog No: NBP3-25795

Method: Colorimetric method

Specification: 100 Assays (Can detect 96 samples without duplication)

Instrument: Spectrophotometer

Sensitivity: 0.26 mg GSH/L

Detection range: 0.26-122.8 mgGSH/L

Average intra-assay CV (%): 1.8

Average inter-assay CV (%): 2.4

Average recovery rate (%): 102

▲ This kit is for research use only.

▲ Instructions should be followed strictly, changes of operation may result in unreliable results.

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

General information

▲Intended use

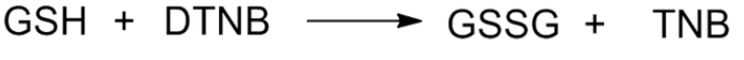
This kit can measure GSH content in serum, plasma, cells and tissue samples.

▲Background

Reduced glutathione (GSH) is a tripeptide which composed of glutamic acid, glycine and cysteine. It is a kind of low molecular scavenger, which can remove O_2^-, H_2O_2, and LOOH. Beside as the main thiol compound of non-protein in the organization, GSH is the substrate of GSH-Px and GST which is indispensable to decomposing hydrogen peroxide for the two enzyme. What's more, 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 vitamin E 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.

▲Detection principle

Reduced glutathione (GSH) can react with dithionitrobenzoic acid (DTNB) to produce thio-nitrobenzoic acid and glutathione disulfide. Nitromercaptobenzoic acid is a yellow compound which has the maximum absorption peak at 420 nm. The GSH content can be calculated by measuring the OD value at 420 nm.the reduced GSH content indirectly.



▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Acid Reagent	45 mL × 2 vials	2-8℃ ,12 months, shading light
Reagent 2	Phosphate	Powder × 2 vials	2-8℃ ,12 months
Reagent 3	DTNB Solution	30 mL × 1 vial	2-8℃ ,12 months, shading light
Reagent 4	Salt Reagent	Powder × 4 vials	2-8℃ ,12 months, shading light
Reagent 5	GSH Standard	3.07 mg × 2 vials	2-8℃ ,12 months
Reagent 6	GSH Standard Stock Diluent	6 mL× 1 vial	2-8℃ ,12 months
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

Spectrophotometer (420 nm), Micropipettor, Vortex mixer

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 point of the assay

The supernatant after centrifugation must be clarified.

Pre-assay preparation

▲Reagent preparation

1. Preparation of reagent 2 application solution
   Dissolve reagent 2 powder with 75 mL ddH_2O completely. It can be stored at 4℃ for 6 months. The reagent is a saturated solution. If there is crystallization, please take the supernatant for experiment.
2. Preparation of reagent 4 application solution
   Dissolve a vial of reagent 4 powder with 10 mL ddH_2O completely. It can be stored at 4℃ for a month with shading light.
3. Preparation of reagent 6 application solution
   Dilute reagent 6 with ddH2O at a rate of 1: 9 and mix fully. Prepare the fresh solution before use.
4. Preparation of 1 mmol/L GSH standard solution

   Dissolve a vial of reagent 5 with 10 mL of reagent 6 application solution fully. Prepare the fresh solution before use.The unused solution can be aliquoted into smaller quantities and stored at -20℃ for 1 month.
5. Preparation of 20 μmol/L standard solution
   Dilute 1 mmol/L GSH standard solution with reagent 6 application solution at a rate of 1: 49 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 pre-experiment and the detection range (0.26-122.8 mgGSH/L).

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

Sample type	Dilution factor
Human serum	1
Mouse serum	1
10% Mouse brain tissue homogenization	1
10% Rat liver tissue homogenization	1
Human plasma	1
Rat plasma	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

▲Detailed operation steps

1. The pretreatment of sample: take 0.7 mL of sample and add 0.7 mL of reagent 1, then centrifuge at 4500 g for 10 min and collect the supernatant for measurement (Please transfer the supernatant to a new EP tube and centrifuge again if the supernatant contain sediments).
2. Blank tube: Add 1 mL of reagent 1 to the 5 mL EP tubes.
   Standard tube: Add 1 mL of 20 μmol/L GSH standard solution to the 5 mL EP tubes.
   Sample tube: Add 1 mL of supernatant to the 5 mL EP tubes.
3. Add 1.25 mL of reagent 2 application solution, 0.25 mL of reagent 3 and 0.05 mL of reagent 4 application solution to each tube.
4. Mix fully and stand for 15 min at room temperature. Set spectrophotometer to zero with double distilled water and measure the OD values of each tube at 420 nm with 1 cm optical path cuvette.

▲Summary operation table

	Blank tube	Standard tube	Sample tube
Reagent 1 (mL)	1		
20 μmol/L GSH standard solution (mL)		1	
Supernatant (mL)			1
Reagent 2 application solution (mL)	1.25	1.25	1.25
Reagent 3 (mL)	0.25	0.25	0.25
Reagent 4 application solution (mL)	0.05	0.05	0.05
Mix fully and stand for 15 min at room temperature. Set spectrophotometer to zero with double distilled water and measure the OD values of each tube at 420 nm with 1 cm optical path cuvette.			

▲Calculation

1. Serum (plasma) sample:

$$\text{GSH content (mgGSH/L)} = \frac{\Delta A_1}{\Delta A_2} \times c \times M \times 2^* \times f$$

2. Tissue and cells sample:

$$\text{GSH content (mgGSH/gprot)} = \frac{\Delta A_1}{\Delta A_2} \times c \times M \times 2^* \times f \div C_{pr}$$

Note:

ΔA_1: $OD_{Sample} - OD_{Blank}$

ΔA_2: $OD_{Standard} - OD_{Blank}$

c: Concentration of standard, 20×10^{-3} mmol/L.

M: Molecular weight of GSH, 307.

2*: Dilution factor of sample pretreatment, 2 times.

f: Dilution factor of sample before test.

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

Appendix I Data

▲Example analysis

Take 0.7 mL of 10% mouse spleen tissue homogenate and 0.7 mL of reagent 1, mix fully and centrifuge at 4500 g for 10 min, then take 1 mL of supernatant and carry the assay according to the operation table. The results are as follows:

The average OD value of the sample is 0.087, the average OD value of the blank is 0.017, the average OD value of the standard is 0.117, the concentration of the standard is 20 μmol/L, the concentration of protein in sample is 6.485 gprot/L, and the calculation result is:

$$\text{GSH content (mgGSH/gprot)} = \frac{0.087-0.017}{0.117-0.017} \times 0.02 \times 307 \times 2 \div 6.485$$

$$=1.34 \text{ (mgGSH/gprot)}$$

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 homogenization medium at 2-8℃ to remove blood cells. 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. Meanwhile, determine the protein concentration of supernatant. 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 homogenization medium 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. Meanwhile, determine the protein concentration of supernatant. If not detected on the same day, the cells sample (without homogenization) can be stored at-80℃ for a month.

Note:

1. Homogenized medium: Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4).
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).

Appendix III References

1. Meister A, Anderson M E. Glutathione. Annu Rev Biochem,1983, 52(6): 711-760.
2. Meister A. Metabolism and function of glutathione: an overview. Biochemical Society Transactions, 1982, 10(2): 78-79.
3. Mannervik B. Glutathione Peroxidase. Methods Enzymol, 1985, 77(5): 490-495.
4. Meister A. Glutathione metabolism and its selective modification. Journal of Biological Chemistry, 1988, 263(33): 17205-17208.