（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K030-S

Specification：50 Assays (48 samples)/ 100 Assays (96 samples)

Measuring instrument：Spectrophotometer（420 nm）

Detection range：0.26-122.8mg GSH/L

Elabscience® Reduced Glutathione (GSH) Colorimetric Assay Kit

This manual must be read attentively and completely before using this product.

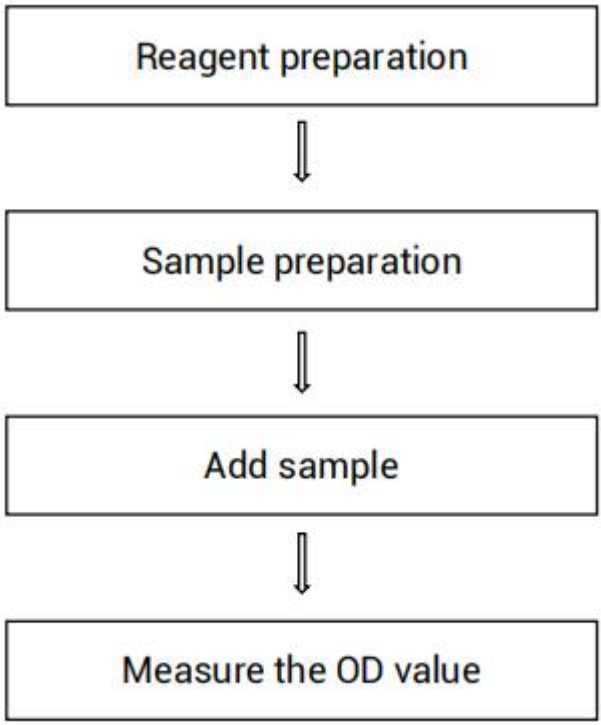
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

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

Table of contents

Assay summary

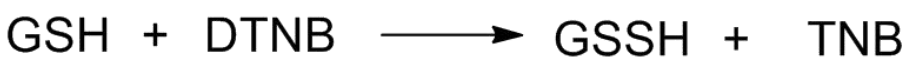


Intended use

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

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.



Kit components & storage

Item	Component	Size 1 (50 assays)	Size 2 (100 assays)	Storage
Reagent 1	Acid Reagent	45 mL × 1 vial	45 mL × 2 vials	2-8℃, 12 months shading light
Reagent 2	Phosphate	Powder × 1 vial	Powder × 2 vials	2-8℃, 12 months
Reagent 3	DTNB Solution	15 mL × 1 vial	30 mL × 1 vial	2-8℃, 12 months shading light
Reagent 4	Salt Reagent	Powder × 2 vials	Powder × 4 vials	2-8℃, 12 months shading light
Reagent 5	GSH Standard	3.07 mg × 1 vial	3.07 mg × 2 vials	2-8℃, 12 months
Reagent 6	GSH Standard Stock Diluent	6 mL× 1 vial	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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

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)

Reagent preparation

① Equilibrate other reagents to room temperature before use.

② The preparation of phosphate application solution：
Dissolve one vial of phosphate with 75 mL double distilled water completely. Store at 4℃ for 6 months. The reagent is a saturated solution. If there is crystallization, please take the supernatant for experiment.

③ The preparation of salt reagent application solution：
Dissolve one vial of salt reagent with 10 mL of double distilled water. Mix well to dissolve. Store at 4°C for 1 month protected from light.

④ The preparation of GSH standard stock application solution：
Dilute 100 uL of GSH standard stock diluent with 900 uL of double-distilled water. The GSH standard diluent should be prepared on spot.

⑤ The preparation of 1 mmol/L GSH standard solution：
Dissolve one vial of GSH standard with 10 mL of GSH standard stock application solution. Mix well to dissolve. The 1 mmol/L GSH standard solution should be prepared on spot. Aliquoted storage at -20°C for 1 month.

⑥ The preparation of 20 μmol/L standard solution：

For each tube, prepare 1 mL of 20 μmol/L standard solution (mix well 20 uL of 1 mmol/L GSH standard solution and 980 uL of GSH standard stock application solution). The 20 μmol/L standard solution should be prepared on spot.

Sample preparation

① Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80℃ for a month.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL normal saline or PBS (0.01 M, pH 7.4) with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Cells:

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 1×10^6 cells in 300-500 μL normal saline(0.9% NaCl) or PBS (0.01 M, pH 7.4) with a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② **Dilution of sample**

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) For the dilution of other sample types, please do pretest to confirm the dilution factor

The key points of the assay

The supernatant after centrifugation must be clarified.

Operating steps

① The pretreatment of sample: take 0.7 mL of sample, add 0.7 mL of acid reagent and mix well. Centrifuge at 4500×g for 10 min. Collect supernatant for detection (Please transfer the supernatant to a new EP tube and centrifuge again if the supernatant contain sediments).

② Blank tube: add 1 mL of acid reagent 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: 1 mL of supernatant to the 5 mL EP tubes.

③ Add 1.25 mL of phosphate application solution, 0.25 mL of DTNB solution and 0.05 mL of salt reagent application solution to each tube.

④ Mix well 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

The sample:

1. Serum (plasma) and other liquid 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 Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (mg GSH/L)	3.60	37.50	76.20
%CV	2.3	1.9	1.2

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean(mg GSH/L)	3.60	37.50	76.20
%CV	2.8	2.0	2.4

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 102%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (mg GSH/L)	16	45.5	85.4
Observed Conc. (mg GSH/L)	16.5	45.5	88.0
Recovery rate (%)	103	100	103

Sensitivity

The analytical sensitivity of the assay is 0.26 mg GSH/L. 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.

Appendix Π Example Analysis

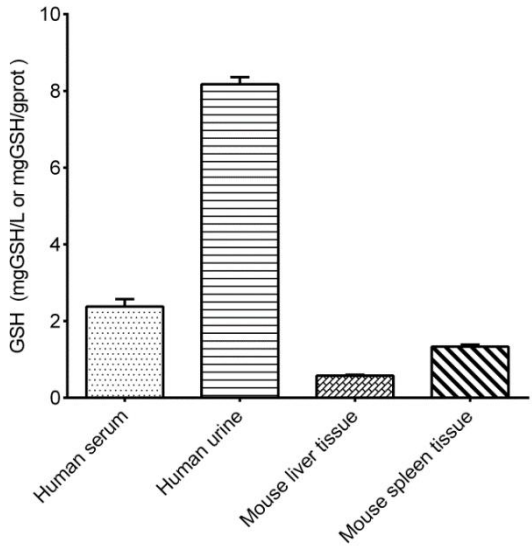
Example analysis：

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

$$\text{GSH content (mgGSH/gprot)} = \frac{0.087 - 0.017}{0.117 - 0.017} \times 0.02 \times 307 \times 2 \div 6.485 = 1.34 \text{ (mgGSH/gprot)}$$

Detect human serum, human urine, 10% mouse liver tissue homogenate (the concentration of protein in sample is 12.68 gprot/L), 10% mouse spleen tissue homogenate (the concentration of protein in sample is 6.49 gprot/L) according to the protocol, the result is as follows：



Appendix III Publications

1. Zhou L , Zhong Y , Li C ,et al.MAPK14 as a key gene for regulating inflammatory response and macrophage M1 polarization induced by ferroptotic keratinocyte in psoriasis[J].Inflammation, 2024, 47(5):1564-1584.DOI:10.1007/s10753-024-01994-8.
2. Mosalam E M , Elberri A I , Abdallah M S ,et al.Mechanistic Insights of Neuroprotective Efficacy of Verapamil-Loaded Carbon Quantum Dots against LPS-Induced Neurotoxicity in Rats[J].International Journal of Molecular Sciences, 2024, 25(14).DOI:10.3390/ijms25147790.
3. Negm W A .Anticancer Effect of Cycas media: Molecular Basis Through Modulation of PI3K/AKT/mTOR Signaling Pathway[J].Molecules, 2024,29.DOI:10.3390/molecules29215013.
4. Abosharaf H A , Gebreel D T , Allam S ,et al.Ehrlich ascites carcinoma provokes renal toxicity and DNA injury in mice: Therapeutic impact of chitosan and maitake nanoparticles[J].Basic & Clinical Pharmacology & Toxicology, 2024, 134(4):13.DOI:10.1111/bcpt.13988.
5. Hacimuftuoglu A , Yesilyurt F , Yilmaz A ,et al.Syringic acid guards against indomethacin-induced gastric ulcer by alleviating inflammation, oxidative stress and apoptosis[J]. 2024.

Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.