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

Catalog No：E-BC-K030-M

Specification：48T(31 samples)/96T(79 samples)/ 500Assays (483 samples)

Measuring instrument：Microplate reader(405-414 nm)

Detection range：2-100 μmol/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
Tel: 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.

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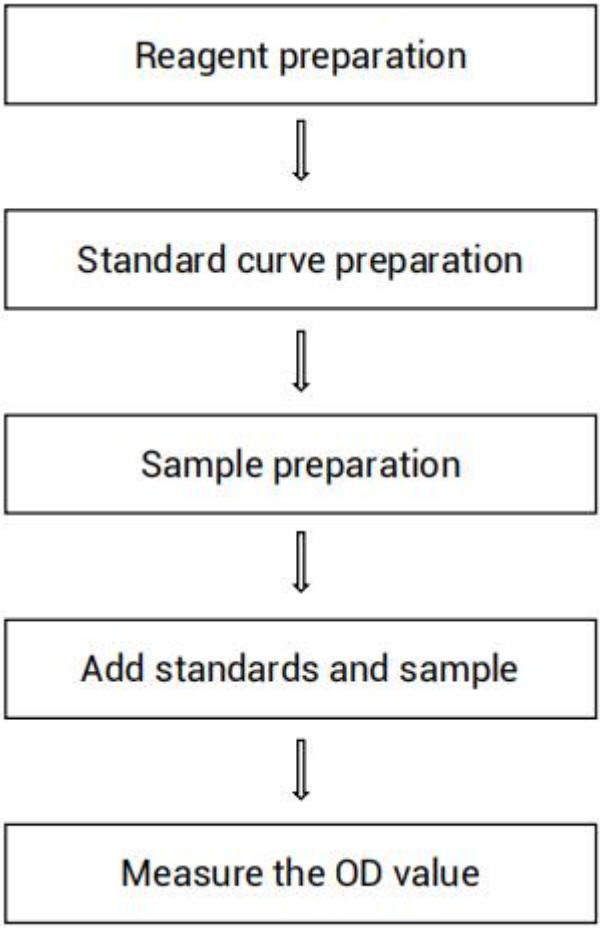
Assay summary

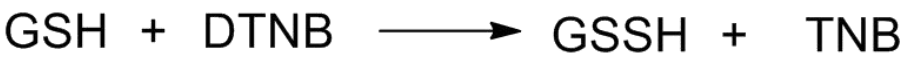


Intended use

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

Detection principle

Reduced Reduced Glutathione (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	Size 1(48 T)	Size 2(96 T)	Size 3 (500Assays)	Storage
Reagent 1	Acid Reagent	6 mL × 1 vial	12 mL × 1 vial	60 mL× 1 vial	2-8℃, 12 months shading light
Reagent 2	Phosphate	6 mL × 1 vial	12 mL × 1 vial	60 mL× 1 vial	2-8℃, 12 months
Reagent 3	DTNB Solution	1.5 mL× 1 vial	1.5 mL×2 vials	15 mL × 1 vial	2-8℃, 12 months shading light
Reagent 4	GSH Standard	3.07 mg×1 vial	3.07 mg×2 vials	3.07 mg×10vials	2-8℃, 12 months
Reagent 5	GSH Standard Stock Diluent	1.5 mL× 1 vial	1.5 mL× 2 vials	15 mL× 1 vial	2-8℃, 12 months
	Microplate	48 wells	96 wells	/	No requirement
	Plate Sealer	2 pieces			
	Sample Layout Sheet	1 piece			

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:

Microplate reader(405-414 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 all reagents to room temperature before use.

② The preparation of GSH standard diluent：
Dilute 1.3 mL of GSH standard stock diluent with 11.7 mL of double-distilled water. The GSH standard diluent should be prepared on spot.

③ The preparation of 1 mmol/L GSH standard solution：
Dissolve 3.07 mg of GSH standard with 10 mL of GSH standard diluent. Mix well to dissolve. The 1 mmol/L GSH standard solution should be prepared on spot. Aliquoted storage at -20°C for 1 month.

④ The preparation of standard curve：
Always prepare a fresh set of standards. Discard working standard dilutions after use.
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. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**10**	**20**	**40**	**50**	**60**	**80**	**100**
1 mmol/L GSH standard (μL)	0	3	6	12	15	18	24	30
GSH standard diluent (μL)	300	297	294	288	285	282	276	270

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 (0.9% NaCl) 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) with a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 10000×g for 10 minutes 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
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). For the dilution of other sample types, please do pretest to confirm the dilution factor

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.

Operating steps

① Preparation of sample supernatant: take 0.1 mL of sample, add 0.1 mL of acid reagent and mix well. Centrifuge at 4500×g for 10 min. Collect supernatant for detection.

② Add 25 μL of DTNB solution to each tube.

③ Control well: add 100 μL of acid reagent.

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

Sample well: add 100 μL of supernatant.

④ Add 100 μL of phosphate solution to each tube.

⑤ Mix well 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

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard # ①) 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).

The sample:

1. Serum (plasma) and other liquid sample:

$$\text{GSH content (μmol/L)} = (\Delta A405\text{-}b) \div a \times 2* \times f$$

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]

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

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

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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 (μmol/L)	5.50	26.40	64.50
%CV	2.3	2.0	1.4

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 (μmol/L)	5.50	26.40	64.50
%CV	3.5	2.9	3.2

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 96%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (μmol/L)	15	45	58
Observed Conc. (μmol/L)	14.9	42.3	55.1
Recovery rate (%)	99	94	95

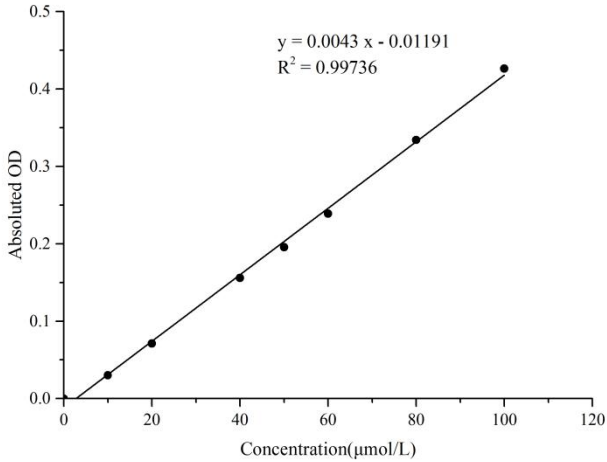
Sensitivity

The analytical sensitivity of the assay is 2 μmol/L GSH 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.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only：

Concentration (μmol/L)	0	10	20	40	50	60	80	100
Average OD	0.057	0.088	0.129	0.213	0.296	0.296	0.392	0.484
Absoluted OD	0.000	0.030	0.071	0.156	0.196	0.239	0.334	0.426



Appendix Π Example Analysis

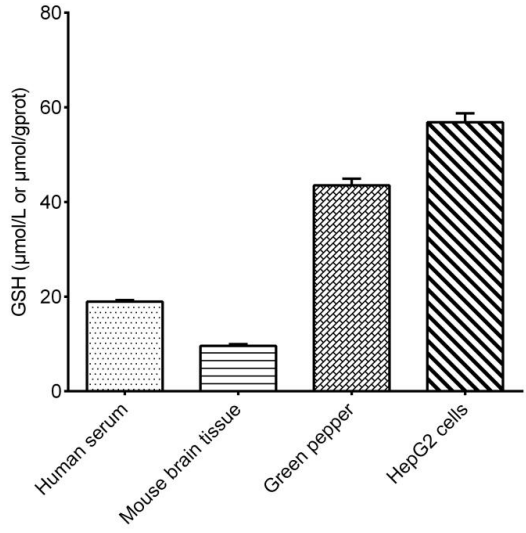
Example analysis：

Take 0.1 mL of human serum sample, add 0.1 mL of acid reagent, mix fully and centrifuge at 4500 g for 10 min, then take prepared supernatant and carry the assay according to the operation steps. 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 control is 0.047, and the calculation result is:

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

Detect human serum, 10% mouse brain tissue homogenate (the concentration of protein is 5.37 gprot/L), 10% green pepper tissue homogenate (the concentration of protein is 1.10 gprot/L), HepG2 cells (the concentration of protein is 3.19 gprot/L) according to the protocol, the result is as follows：



Appendix III Publications

1. Zang X , He X Y , Xiao C M ,et al.Circular RNA-encoded oncogenic PIAS1 variant blocks immunogenic ferroptosis by modulating the balance between SUMOylation and phosphorylation of STAT1[J].Molecular Cancer, 2024, 23(1):1-19.DOI:10.1186/s12943-024-02124-6.
2. Liu J , Chang Y , Zhou W ,et al.An esterase-activatable nanoprodrug mitigates severe spinal cord injury via alleviating ferroptosis and reprogramming inflammatory microenvironment[J].Nano Today, 2024, 56(000):15.DOI:10.1016/j.nantod.2024.102229.
3. Liu H, Ji M, Qin Y, et al. Harnessing self-assembled nanoplatform of Dexamethasone and α-linolenic acid for high-efficiency inhibition of pulmonary cytokine storm and fibrosis in mice[J]. Nano Today, 2024, 55: 102201.
4. Qiu C, Tang C, Tang Y, et al. RGS5+ lymphatic endothelial cells facilitate metastasis and acquired drug resistance of breast cancer through oxidative stress-sensing mechanism[J]. Drug Resistance Updates, 2024, 77: 101149.
5. Wang Y, Liang X, Andrikopoulos N, et al. Remediation of Metal Oxide Nanotoxicity with a Functional Amyloid[J]. Advanced Science, 2024, 11(23): 2310314.
6. Zhang L , Shi W Y ,Jia-Ying XuYan LiuShi-Jia WangJia-Yang ZhengYun-Hong LiLin-Xi YuanLi-Qiang Qin.Protective effects and mechanism of chemical- and plant-based selenocystine against cadmium-induced liver damage[J].Journal of Hazardous Materials, 2024, 468(Apr.15):133812.1-133812.12.DOI:10.1016/j.jhazmat.2024.133812.

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.