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

Catalog No：E-BC-K097-S

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

Measuring instrument：Spectrophotometer（412 nm）

Detection range：0.12-30 μmol/L T-GSH

Elabscience®Total Glutathione (T-GSH)/Oxidized Glutathione (GSSG) 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.

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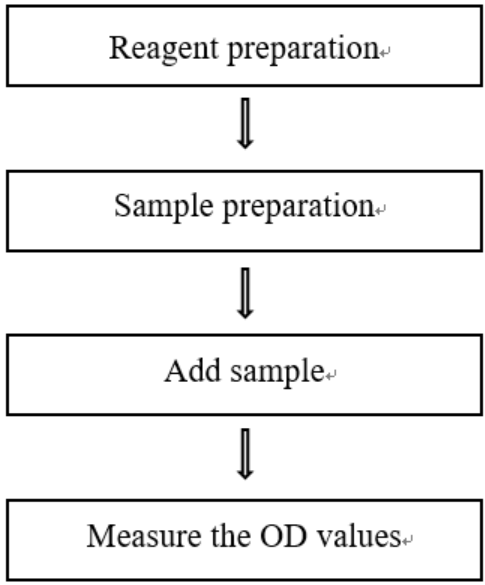
Assay summary



Intended use

This kit can be used to measure total glutathione (T-GSH) and oxidized glutathione (GSSG) content in serum, plasma, animal tissue, whole blood, red blood cells and cultured cells samples.

Detection principle

GSSG is reduced to GSH by glutathione reductase, and GSH can react with DTNB to produce GSSG and yellow TNB. The amount of total glutathione (GSSG+GSH) determines the amount of yellow TNB. Thus the total glutathione can be calculated by measuring the OD value at 412 nm. The content of GSSG can be determined by first removing GSH from the sample with appropriate reagent and then using the above reaction principle.

Kit components & storage

Item	Component	Size 1 (50 assays)	Size 2 (100 assays)	Storage
Reagent 1	Buffer Solution	55 mL ×2 vials	55 mL ×3 vials	2-8℃, 12 months
Reagent 2	GSSG Standard	6.13 mg ×1 vial	6.13 mg ×1 vial	-20℃, 12 months
Reagent 3	Protein Precipitator	60 mL ×1 vial	60 mL ×2 vials	-20℃, 12 months
Reagent 4	Enzyme Stock Solution	77 μL × 1 vial	154 μL × 1 vial	-20℃, 12 months
Reagent 5	Chromogenic Agent	Powder ×1 vial	Powder ×2 vials	2-8℃, 12 months, shading light
Reagent 6	Diluent	1.8 mL ×1 vial	3.6 mL ×1 vial	2-8℃, 12 months
Reagent 7	GSH Scavenger Auxiliary Solution	0.7 mL ×1 vial	1.4 mL ×1 vial	2-8℃, 12 months
Reagent 8	GSH Scavenger	0.1 mL ×1 vial	0.2 mL ×1 vial	-20℃, 12 months, shading light
Reagent 9	Substrate	Powder ×1 vial	Powder ×2 vials	-20℃, 12 months, shading light
Reagent 10	Stop Solution	25 mL ×1 vial	50 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 (412 nm), Micropipettor, Incubator, Vortex mixer, Centrifuge

Reagents:

Double distilled water, PBS (0.01 M, pH 7.4), Absolute ethanol

Reagent preparation

① Keep enzyme stock solution on ice during use. Equilibrate other reagents to room temperature before use.

② The preparation of 1 mmol/L GSSG standard stock solution:
Dissolve one vial of GSSG standard with 10 mL of double distilled water, mix well to dissolve. Aliquoted storage at -20 ℃ for 1 month.

③ The preparation of 8 μmol/L GSSG standard solution:
Before testing, please prepare sufficient 8 μmol/L GSSG standard solution according to the test wells. For example, prepare 125 μL of 8 μmol/L GSSG standard solution (mix well 1 μL of 1 mmol/L GSSG standard stock solution and 124 μL of protein precipitator). Store at 2-8 ℃ for 24 h.

④ The preparation of enzyme stock working solution:
Dilute 5 μL of enzyme stock solution with 95 μL of buffer solution, mix well. Store at 2-8 ℃ for 24 h.

⑤ The preparation of chromogenic working solution:
Dissolve one vial of chromogenic agent with 1.5 mL of diluent, mix well to dissolve. Aliquoted storage at -20 ℃ for 3 months.

⑥ The preparation of reaction working solution：

Before testing, please prepare sufficient reaction working solution according to the test wells. For example, prepare 810 μL of reaction working solution (mix well 30 μL of enzyme stock working solution, 30 μL of chromogenic working solution and 750 μL of buffer solution). Store at 2-8 ℃ for 24 h.

⑦ The preparation of GSH scavenger auxiliary working solution：

Before testing, please prepare sufficient GSH scavenger auxiliary working solution according to the test wells. For example, prepare 40 μL of GSH scavenger auxiliary working solution (dilute 20 μL of GSH scavenger auxiliary solution with 20 μL of double distilled water, mix well). Store at 2-8 ℃ for 24 h.

⑧ The preparation of GSH scavenger working solution：

Before testing, please prepare sufficient GSH scavenger working solution according to the test wells. For example, prepare 50 μL of GSH scavenger working solution (dilute 5 μL of GSH scavenger with 45 μL of absolute ethanol, mix well). Store at 2-8 ℃ for 24 h.

⑨ The preparation of substrate stock solution：

Dissolve one vial of substrate with 150 μL of double distilled water, mix well to dissolve. Aliquoted storage at -70 ℃ for 3 months.

⑩ The preparation of substrate working solution：

Before testing, please prepare sufficient substrate working solution according to the test wells. For example, prepare 400 μL of substrate working solution (mix well 5 μL of substrate stock solution with 395 μL of buffer solution). Store at 2-8 ℃ for 24 h.

Sample preparation

① Sample preparation

Serum and plasma:

① Prepare serum/plasma as the common method.

② Take 100 μL of sample and add 400 μL of protein precipitator, mix fully by a vortex mixer for 30 s, stand for 5 min at 4℃.

③ Centrifuge at 3100×g for 10 min.

④ Take the supernatant and preserve it on ice for detection.

Whole blood:

① Collect blood, use heparin or EDTA as the anticoagulation.

② Take 100 μL of whole blood and add 400 μL of protein precipitator, mix fully for 30 s with a vortex mixer, stand for 5 min at 4℃.

③ Centrifuge at 3100×g for 10 min.

④ Take the supernatant and preserve it on ice for detection.

Red blood cell:

① Collect blood, use heparin or EDTA as the anticoagulation.

② Centrifuge at 2000 rpm for 10 min immediately, remove the plasma and leukocytic layer (upper layer) carefully.

③ Take 100 μL of red blood cell, add 400 μL of protein precipitator, mix fully for 30 s with a vortex mixer, stand for 5 min at 4℃.

④ Centrifuge at 3100×g for 10 min.

⑤ Take the supernatant and preserve it on ice for detection.

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 protein precipitator with a dounce homogenizer at 4℃.

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

Cell (adherent or suspension) samples:

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 400 μL protein precipitator with a ultrasonic cell disruptor at 4℃.

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

② Dilution of sample

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

Sample type	Dilution factor
Human serum	1
Rat plasma	1
Mouse serum	1
10% Rat heart tissue homogenate	10
10% Rat liver tissue homogenate	60
10% Rat brain tissue homogenate	10

Note: The diluent is protein precipitator. For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① The viscosity of GSH scavenger auxiliary solution is very high, so should be pipetted slowly slowly and carefully.

② The GSH scavenger has a pungent odor. Please operate in the fume hood.

Operating steps

The measurement of T-GSH

① Blank tube: take 40 μL of protein precipitator to the 2 mL EP tube

Standard well: take 40 μL of 8 μmol/L GSSG standard solution to the 2 mL EP tube.

Sample well: take 40 μL of pretreated sample to the 2 mL EP tube.

② Add 600 μL of reaction working solution to each tube and incubate at room temperature or 25℃ for 5 min.

③ Add 200 μL of substrate working solution to each tube, mix fully for 5 s with vortex mixer.

④ Incubate at room temperature or 25℃ for 25 min and add 400 μL of stop solution to each tube.

⑤ Set the spectrophotometer to zero with double distilled water and measure the OD values of each tube at 412 nm wavelength with 0.5 cm optical path cuvette.

The measurement of GSS

① Pretreatment of solution for blank tube: Add 20 μL of GSH scavenger auxiliary working solution to 100 μL of protein precipitator, mix fully with a vortex mixer, then take 100 μL of liquid to 0.5 mL EP tube and add 4 μL of GSH scavenger working solution, mix fully with a vortex mixer immediately, react at 25℃ for an hour.

② Pretreatment of standard: Add 20 μL of GSH scavenger auxiliary working solution to 100 μL of 8 μmol/L GSSG standard solution, mix fully with a vortex mixer, then take 100 μL of liquid to 0.5 mL EP tube and add 4 μL of GSH scavenger working solution, mix fully with a vortex mixer immediately, react at 25℃ for an hour.

③ Remove the GSH of samples (for sample tube): Add 20 μL of GSH scavenger auxiliary working solution to 100 μL of pretreated sample in sample preparation

step, mix fully with a vortex mixer, then take 100 μL of liquid to 0.5 mL EP tube and add 4 μL of GSH scavenger working solution, mix fully with a vortex mixer immediately, react at 25℃ for an hour.

④ Blank tube of GSSG: take 40 μL of pretreated blank solution to the 2 mL EP tube.

Standard tube of GSSG: take 40 μL of pretreated standard to the 2 mL EP tube.

Sample tube of GSSG： take 40 μL of sample supernatant to the 2 mL EP tube

⑤ Add 600 μL of reaction working solution to each tube and incubate at room temperature or 25℃ for 5 min.

⑥ Add 200 μL of substrate working solution to each tube, mix fully for 5 s with vortex mixer.

⑦ Incubate at room temperature or 25℃ for 25 min and add 400 μL of stop solution to each tube.

⑧ Set the spectrophotometer to zero with double distilled water and measure the OD values of each tube at 412 nm wavelength with 0.5 cm optical path cuvette.

Calculation

The sample:

1. Serum (plasma), whole blood, red blood cells samples:

$$\text{T-GSH content (μmol/L)} = \frac{A_1 - A_0}{A_2 - A_0} \times c_1 \times 5^* \times f_1$$

$$\text{GSSG content (μmol/L)} = \frac{A_4 - A_3}{A_5 - A_3} \times c_2 \times 5^* \times f_2$$

2. Tissue sample:

$$\text{T-GSH content (μmol/kg)} = \frac{A_1 - A_0}{A_2 - A_0} \times c_1 \div \frac{m}{V_1} \times f_3$$

$$\text{GSSG content (μmol/kg)} = \frac{A_4 - A_3}{A_5 - A_3} \times c_2 \div \frac{m}{V_1} \times f_4$$

3. Cultured cells samples:

$$\text{T-GSH content } (\mu\text{mol}/10^9) = \frac{A_1 - A_0}{A_2 - A_0} \times c_1 \div \frac{n^{**}}{V_2} \times f_5$$

$$\text{GSSG content } (\mu\text{mol}/10^9) = \frac{A_4 - A_3}{A_5 - A_3} \times c_2 \div \frac{n^{**}}{V_2} \times f_6$$

Reduced GSH content = T-GSH content - 2 ×GSSG content

[Note]

A_0: OD_{Blank} of T-GSH.

A_1: OD_{Sample} of T-GSH.

A_2: $OD_{Standard}$ of T-GSH.

A_3: OD_{Blank} of GSSG.

A_4: OD_{Sample} of GSSG.

A_5: $OD_{Standard}$ of GSSG.

c_1: 16 μmol/L (When converting GSSG to GSH as a standard, multiply by 2).

c_2: 8 μmol/L GSSG.

5*: Dilution multiple of sample in sample pretreatment step.

f_1: Dilution factor of sample before test when measure T-GSH for serum (plasma), whole blood, red blood cells samples.

f_2: Dilution factor of sample before test when measure GSSG for serum (plasma), whole blood, red blood cells samples.

m: the fresh weight of sample.

V_1: the volume of protein precipitator in sample preparation step of tissue sample.

f_3: Dilution factor of sample before test when measure T-GSH for animal tissue.

f_4: Dilution factor of sample before test when measure GSSG for animal tissue.

n**: When the cell number is 1×10^6, n=1.

V_2: The volume of protein precipitator in sample preparation step of cell sample.

f_5: Dilution factor of sample before test when measure T-GSH for cultured cells samples.

f_6: Dilution factor of sample before test when measure GSSG for cultured cells samples.

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)	3.50	16.40	23.50
%CV	1.2	0.9	0.6

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)	3.50	16.40	23.50
%CV	4.5	4.7	4.9

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 97%.

	Sample 1	Sample 2	Sample 3
Expected Conc. （μmol/L）	6.8	17.5	25
Observed Conc. （μmol/L）	6.7	16.8	24.0
recovery rate(%)	99	96	96

Sensitivity

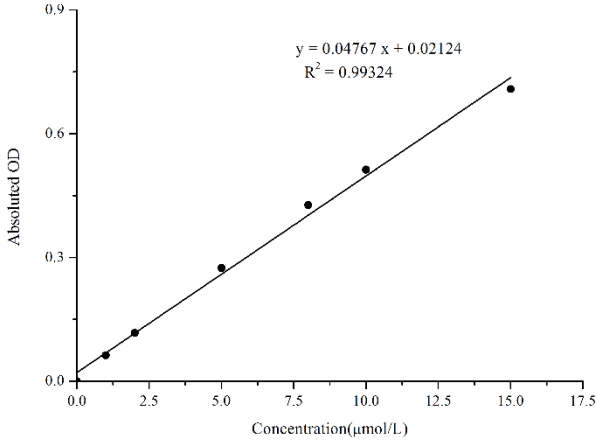
The analytical sensitivity of the assay is 0.12 μmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

(It doesn't need to prepare the standard curve for this kit and the provided standard curve is for reference only)

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**	**1**	**2**	**5**	**8**	**10**	**15**
Average OD	0.020	0.082	0.136	0.294	0.446	0.532	0.728
Absoluted OD	0	0.062	0.116	0.274	0.426	0.512	0.708



Appendix II Example Analysis

Example analysis:

Dilute 10% mouse liver tissue homogenate with reagent 3 for 60 times, take 40 μL of diluted sample for the measurement of T-GSH and take 100 μL of of diluted sample for the measurement of GSSG, carry the assay according to the operation table. The results are as follows:

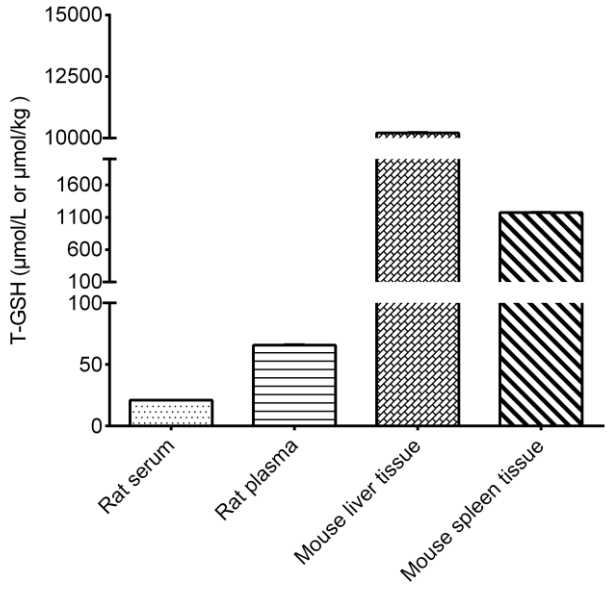
The results of T-GSH: the average OD value of the blank is 0.022, the average OD value of the standard is 0.354, the average OD value of the sample is 0.414, and the calculation result is:

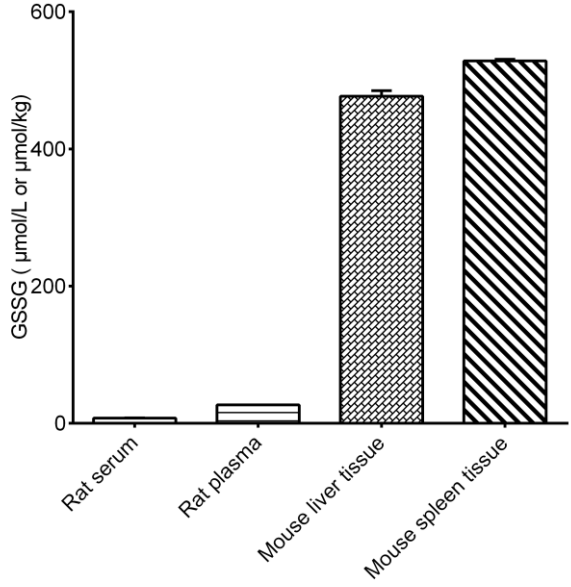
$$\text{T-GSH (μmol/kg)} = \frac{(0.414-0.022)}{(0.354-0.022)} \times 16 \times 60 \div 0.05 \times 0.45 = 10201.45 \text{ μmol/kg}$$

The results of GSSG: the average OD value of the blank is 0.025, the average OD value of the standard is 0.330, the average OD value of the sample is 0.059, and the calculation result is:

$$\text{GSSG (μmol/kg)} = \frac{(0.059-0.025)}{(0.330-0.025)} \times 8 \times 60 \div 0.05 \times 0.45 = 481.57 \text{ μmol/kg}$$

Detect rat serum, rat plasma, 10% mouse liver tissue homogenate (dilute for 60 times), 10% mouse spleen tissue homogenate (dilute for 10 times) according to the protocol, the result is as follow


T-GSH (μmol/L or μmol/kg)
15000
12500
10000
1600
1100
600
100
100
50
0
Rat serum
Rat plasma
Mouse liver tissue
Mouse spleen tissue


GSSG (μmol/L or μmol/kg)
600
400
200
0
Rat serum
Rat plasma
Mouse liver tissue
Mouse spleen tissue

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.