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Catalog No：E-BC-F045

Specification：96T(40 samples)

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=390 nm/490 nm)

Detection range：9.16-1000 μmol/L

Elabscience® Total Glutathione (T-GSH) And Reduced Glutathione (GSH) Fluorometric 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

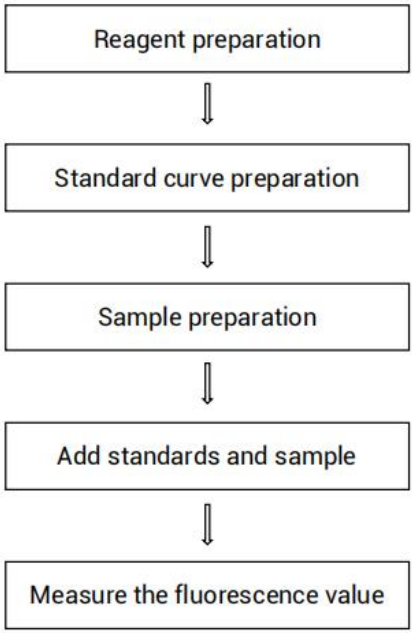
Email: techsupport@elabscience.com

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

The kit is suitable for detecting the content of total glutathione (T-GSH) and reduced glutathione (GSH) in serum, plasma, animal tissues, and cells.

Detection principle

Glutathione is a thiol-containing tripeptide compound widely distributed in organisms, participating in various important physiological processes. It protects cells from oxidative damage and detoxifies drug metabolites. Glutathione exists in cells in two forms: reduced glutathione (GSH) and oxidized glutathione (GSSG).

GSH can react with monochlorobimane to generate a fluorescent compound. The measured fluorescence value is then substituted into a standard curve to calculate the GSH content. After adding a reducing reagent, GSSG is reduced to GSH, thereby determining the total glutathione content in the sample.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	50 mL × 1 vial	-20°C, 12months,
Reagent 2	Enzyme Reagent	Powder × 2 vials	-20°C, 12 months, shading light
Reagent 3	Coenzyme	Powder × 2 vials	-20°C, 12 months, shading light
Reagent 4	Probe	0.12 mL × 1 vial	-20°C, 12 months, shading light
Reagent 5	GSH Standard	Powder × 1 vial	-20°C, 12 months, shading light
	Black Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
	Sample Layout Sheet	1 piece	

Note: All the reagents should be stored according to the table. The reagents from different kits can not be mixed or used interchangeably. For liquid reagents with small volumes or powders, centrifuge them before use to prevent loss.

Instruments

Fluorescence microplate reader (Ex/Em=390 nm/490 nm), Incubator

Materials required but not provided

Normal saline (0.9% NaCl), 3 mol/L perchloric acid, 3 mol/L NaOH, Distilled or deionized water, PBS (0.01 M, pH 7.4), pH indicator paper

Reagent preparation

① Equilibrate all the reagents to 25°C before use.

② Enzyme Working Solution preparation:

Dissolve one vial of Enzyme Reagent with 550 μL of distilled or deionized water. Mix well to dissolve. Store at -20°C for 7 days protected from light.

③ Coenzyme Working Solution preparation:

Dissolve one vial of Coenzyme with 550 μL of distilled or deionized water. Mix well to dissolve. Store at -20°C for 7 days protected from light.

④ T-GSH Working Solution preparation:

Before testing, please prepare sufficient T-GSH Working Solution. For example, prepare 500 μL of T-GSH Working Solution (mix well 300 μL of Buffer Solution, 100 μL of Enzyme Working Solution and 100 μL of Coenzyme Working Solution). The T-GSH Working Solution should be freshly prepared before use. Stable for 2 h protected from light.

⑤ GSH Working Solution preparation:

Before testing, please prepare sufficient GSH Working Solution. For example, prepare 500 μL of GSH Working Solution (mix well 400 μL of Buffer Solution and 100 μL of Coenzyme Working Solution). The GSH Working Solution should be freshly prepared before use. Stable for 2 h protected from light.

⑥ Chromogenic Working Solution preparation:

Before testing, please prepare sufficient Chromogenic Working Solution. For example, prepare 505 μL of Chromogenic Working Solution (mix well 500 μL of Buffer Solution and 5 μL of Probe). The Chromogenic Working Solution should be freshly prepared before use. Stable for 2 h protected from light.

⑦ 20 mmol/L Standard Solution preparation：

Dissolve one vial of GSH standard with 1 mL of Buffer Solution. Mix well to dissolve. Store at -20°C for one month protected from light.

⑧ 1 mmol/L Standard Solution preparation：

Before testing, prepare a sufficient 1 mmol/L Standard Solution according to the test wells. For example, prepare 1000 μL of 1 mmol/L Standard Solution (mix 950 μL of Buffer Solution and 50 μL of 20 mmol/L Standard Solution thoroughly). The 1 mmol/L Standard Solution should be freshly prepared before use. Stable for 8 h when stored at -20°C protected from light.

⑨ Standard curve preparation：

Always prepare a fresh set of Standards. Discard Working Standard Dilutions after use.

Dilute 1 mmol/L Standard Solution with Buffer Solution to a serial concentration. The recommended dilution gradient is as follows: 0, 25, 50, 100, 200, 400, 800, 1000 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**25**	**50**	**100**	**200**	**400**	**800**	**1000**
1 mmol/L Standard (μL)	0	5	10	20	40	80	160	200
Buffer solution (μL)	200	195	190	180	160	120	40	0

Sample preparation

Serum and plasma:

① Mix 200 μL of serum or plasma and 5 μL of 3 mol/L perchloric acid, vortex at least 30 s immediately. Incubate at 4°C (or on ice) for 5 min.

② Centrifuge at 10000×g for 10 min at 4°C. Collect supernatant and add NaOH solution to adjust the pH to 7-9.5 (test 1 μL of the sample with pH indicator paper).

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 30 mg).

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

③ Homogenize 30 mg tissue in 270 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4°C.

④ Centrifuge at 10000×g for 10 min at 4°C to collect supernatant.

⑤ Mix 160 μL supernatant and 4 μL 3 mol/L perchloric acid, vortex at least 30 s immediately. Incubate at 4°C (or on ice) for 5 min.

⑥ Centrifuge at 10000×g for 10 min at 4°C to collect supernatant.

⑦ Collect supernatant and add NaOH solution to adjust the pH to 7-9.5 (test 1 μL of the sample with pH indicator paper).

(For mouse liver tissue, the recommended ratio is **45: 1 (Supernatant: 3 mol/L NaOH=45: 1)**. However, the optimal ratio should be adjusted based on the specific sample conditions.)

Cell sample

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 200 μL buffer with a dounce homogenizer at 4°C.

④ Centrifuge at 10000×g for 10 min at 4°C to collect supernatant.

⑤ Mix 160 μL supernatant and 4 μL 3 mol/L perchloric acid, vortex at least 30 s immediately. Incubate at 4°C (or on ice) for 5 minutes.

⑥ Centrifuge at 10000×g for 10 min at 4°C to collect supernatant.

⑦ Collect supernatant and add NaOH solution to adjust the pH to 7-9.5 (test 1 μL of the sample with pH paper).

Dilution of sample

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

Sample type	Dilution factor
10% Mouse kidney tissue homogenization	1
10% Mouse liver tissue homogenization	1
10% Mouse brain tissue homogenization	1
10% Mouse lung tissue homogenization	1
10% Mouse heart tissue homogenization	1
10% Mouse muscle tissue homogenization	1
1×10^6 RAW264.7 cells	1
1×10^6 HL 60 cells	1
1×10^6 Jurkat cells	1

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

The key points of the assay

The probe must be protected from light during use to prevent fluorescence quenching.

Operating steps

① Standard well: Add 10 μL of Standard Solution with different concentrations to the corresponding wells.
T-GSH sample well: Add 10 μL of sample to the corresponding wells.
GSH sample well: Add 10 μL of sample to the corresponding wells.

② Add 50 μL of T-GSH Working Solution to standard wells and T-GSH sample wells.

③ Add 50 μL of GSH Working Solution to GSH sample wells.

④ Incubate at 37°C for 10 min.

⑤ Add 100 μL Chromogenic Working Solution to each well.

⑥ Shake the microplate for 5 seconds to ensure complete mixing. Incubate at 37°C for 20 min. Measure the fluorescence intensity at the excitation wavelength of 390 nm and the emission wavelength of 490 nm.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean fluorescence value of the blank (Standard #①) from all standard readings. This is the net fluorescence intensity.

3. Plot the standard curve by using the net fluorescence intensity 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) sample:

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

2. Tissue sample:

$$\text{T-GSH/GSH content (μmol/g wet weight)} = (\Delta F - b) \div a \times V \div m \times f \div 1000$$

3. Cell sample:

$$\text{T-GSH/GSH content (nmol/10^6)} = (\Delta F - b) \div a \times V \div n \times f \times 1000$$

[Note]

GSSG content = (T-GSH content - GSH content)/2

(GSSG is reduced by reducing agents to 2 × GSH)

ΔF: $\Delta F = F_{sample} - F_{blank}$ (The net fluorescence intensity of the sample well).

m: The weight of tissue, g.

V: The volume of normal saline (0.9% NaCl), L.

n: The number of cell sample/10^6.

f: Dilution factor of sample before tested.

1000: 1 μmol/L = 1000 nmol/L.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three mouse liver 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)	520.3	600	680.6
%CV	2.6	3.2	5

Inter-assay Precision

Three mouse liver 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)	520.3	600	680.6
%CV	5.2	5.0	5.9

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 99.7%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (μmol/L)	520.3	600	680.6
Observed Conc. (μmol/L)	535.9	570.0	687.4
Recovery rate (%)	103	95	101

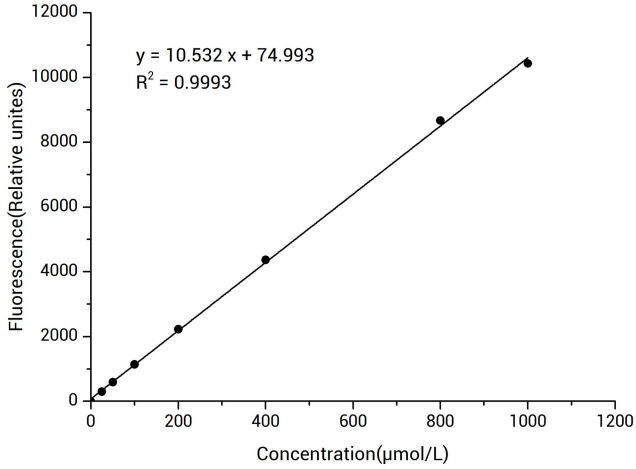
Sensitivity

The analytical sensitivity of the assay is 9.16 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:

As the fluorescence 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**	**25**	**50**	**100**	**200**	**400**	**800**	**1000**
Fluorescene value	1511	1788	2088	2639	3723	5812	10261	11912
	1490	1814	2088	2639	3726	5929	10084	11950
Average fluorescene value	1501	1801	2088	2639	3725	5871	10173	11931
Absoluted fluorescene value	0	300	587	1138	2224	4370	8672	10430



Appendix Π Example Analysis

Example analysis：

Take 10 μL of 10% mouse liver tissue homogenate supernatant to the well of microplate. Proceed according to the operating steps. The results are as follows:

standard curve: y = 10.532 x + 74.993, the average fluorescence value of the blank well is 1501, the fluorescence value of the T-GSH sample well is 9155, $\Delta F_{T\text{-}GSH} = F_{sample} - F_{blank}$ = 9155 - 1501 = 7654. The fluorescence value of the GSH sample well is 4004, $\Delta F_{GSH} = F_{sample} - F_{blank}$ = 4004 - 1500 = 2504, and the calculation result is:

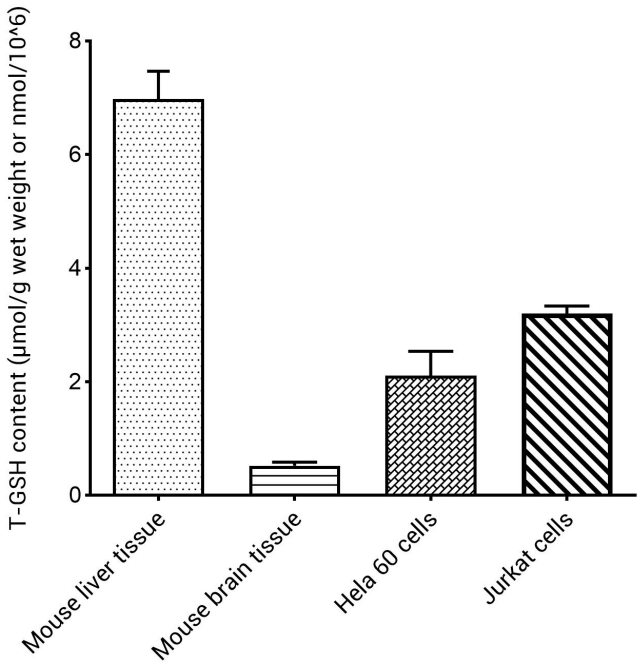
$$\text{T-GSH content (μmol/g wet weight)} = (\,7564 - 74.993\,) \div 10.532 \times 0.9 \div 0.1 \div 1000$$

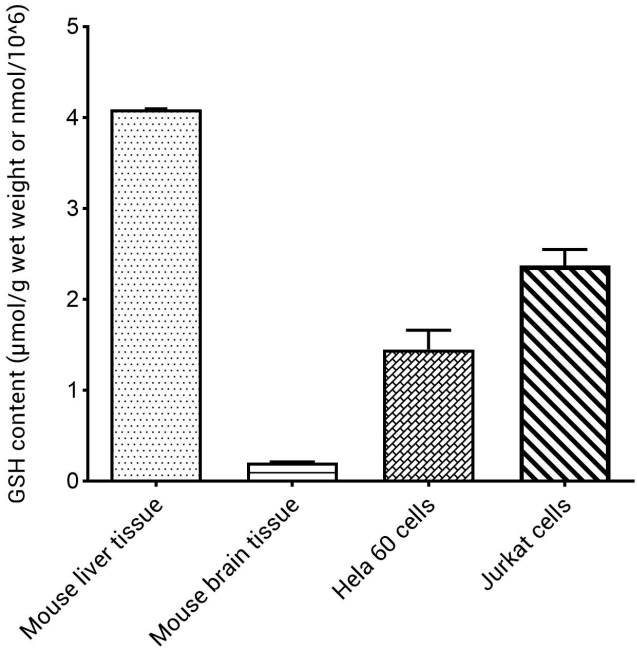
$$= 6.40\ \text{μmol/g wet weight}$$

$$\text{GSH content (μmol/g wet weight)} = (4004 - 74.993\,) \div 10.532 \times 0.9 \div 0.1 \div 1000$$

$$= 3.36\ \text{μmol/g wet weight}$$

Detect 10% mouse liver tissue homogenate supernatant, 10% mouse brain tissue homogenate supernatant, 1×10^6 HL 60 cells and 1×10^6 Jurkat cells according to the protocol, the result is as follows：


T-GSH content (μmol/g wet weight or nmol/10^6)
8
6
4
2
0
Mouse liver tissue
Mouse brain tissue
Hela 60 cells
Jurkat cells


GSH content (µmol/g wet weight or nmol/10^6)
5
4
3
2
1
0
Mouse liver tissue
Mouse brain tissue
Hela 60 cells
Jurkat cells

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.