IMMUNOLOGICAL SCIENCES Product Data Sheet

CO-K097-S Total Glutathione (T-GSH)/Oxidized Glutathione (GSSG) Colorimetric Assay Kit

Size: 100Assays (Can detect 96 samples tor T-GSH or GSSG without duplication, or detect 46 samples tor both T-GSH and GSSG without duplication)

Detection Method: Colorimetric method

Measuring instrument: Spectrophotometer

Assay Time: 90-100 minutes

Sensitivity: 0.12 μmol/L T-GSH

Detection range: 0.12-30 μmol/L T-GSH

Average intra-assay CV (%): 0.9

Average inter-assa y CV(%): 4.7

Average recovery rate (%): 97

Sample Type: Serum, plasma, animal tissue, whole blood, red blood cells, culture cell samples

General information

▲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.

▲Background

Glutathione is a tripeptide (γ-L-glutamyl-L-Cysteinyl glycine) and the most common intracellular thiol. In cells, glutathione exists in two different forms, reduced (GSH) and oxidized (GSSG). Under physiological conditions, more than 98% of intracellular glutathione is GSH, because GSSG is rapidly reduced to GSH by glutathione reductase. GSH-GSSG system is the most abundant oxidation-reduction system in eukaryotic cells. It plays an important role in cell homeostasis and participates in apoptosis-related signal transduction.

▲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	Specification	Storage
Reagent 1	Buffer Solution	55 mL × 3 vials	2-8℃ , 12 months
Reagent 2	GSSG Standard	6.13 mg × 1 vial	-20℃ , 12 months
Reagent 3	Protein Precipitator	60 mL × 2 vials	2-8℃ , 12 months
Reagent 4	Enzyme Stock Solution	154 μL × 1 vial	-20℃ , 12 months
Reagent 5	Chromogenic Agent	Powder × 2 vials	2-8℃ , 12 months, shading light
Reagent 6	Diluent	3.6 mL × 1 vial	2-8℃ , 12 months
Reagent 7	GSH Scavenger Auxiliary Solution	1.4 mL × 1 vial	2-8℃ , 12 months
Reagent 8	GSH Scavenger	0.2 mL × 1 vial	-20℃ , 12 months, shading light
Reagent 9	Substrate	Powder × 2 vials	-20℃ , 12 months, shading light
Reagent 10	Stop Solution	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.

▲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

▲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 points of the assay

1. The viscosity of reagent 7 is very high, so should be pipetted slowly slowly and carefully.
2. The reagent 8 has a pungent odor. Please operate in the fume hood.

Pre-assay preparation

▲Reagent preparation

1. Preparation of reagent 4 working solution:

(Operate on ice) Dilute the reagent 4 with reagent 1 at the ratio of 1:19. Prepare the fresh solution before use and the prepared solution can be stored at 2-8℃ for 24 hours. (Note: Reagent 4 should be mix fully before use.)

2. Preparation of reagent 5 working solution:

Dissolve a vial of reagent 5 with 1.5 mL of reagent 6 fully. Unused reagent 5 working solution can be stored at -20℃ for 3 months. It is recommended to aliquot the prepared solution into small quantities and store at -20℃ .

3. Preparation of reactive working solution:

Mix the reagent 4 working solution, reagent 5 working solution and reagent 1 at the ratio of 1: 1: 25. Prepare the fresh solution before use and the prepared solution can be stored at 2-8℃ for 24 hours.

4. Preparation of reagent 7 working solution:

Dilute the reagent 7 with double distilled water at the ratio of 1: 1. Prepare the fresh solution before use and the prepared solution can be stored at 2-8℃ for 24 hours. (Note: reagent 7 should be pipetted slowly.)

5. Preparation of reagent 8 working solution:

Dilute the reagent 8 with absolute ethanol (self-prepared) at the ratio of 1: 9. Prepare the fresh solution before use and the prepared solution can be stored at 2-8℃ for 24 hours.

6. Preparation of reagent 9 stock solution:

Dissolve a vial of reagent 9 with 150 μL of double distilled water fully. It is recommended to aliquot the prepared solution into small quantities and unused solution can be stored at -70℃ for 3 months.

7. Preparation of reagent 9 working solution:

Dilute the reagent 9 stock solution with reagent 1 at the ratio of 1:79. Prepare the fresh solution before use and the prepared solution can be stored at 2-8℃ for 24 hours.

8. Preparation of 1 mmol/L GSSG standard stock solution:

Dissolve a vial of reagent 2 fully with 10 mL double distilled water. Aliquot the stock solution into small quantities and it can be store at -20℃ for 1 month.

9.Preparation of 8 μmol/L GSSG standard solution:

Dilute 1 mmol/L GSSG standard stock solution with reagent 3 at the ratio of 1:124. Prepare the fresh solution before use and the prepared solution can be stored at 2-8℃ for 24 hours.

▲Sample preparation

Sample requirements

The sample should not contain DTT, 2-mercaptoethanol and other reducing agents.

1. Whole blood

(1) Collect blood, use heparin or EDTA as the anticoagulation.
(2) Take 100 μL of whole blood and add 400 μL of reagent 3 , 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.

2. Red blood cell

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

(2) Centrifuge at 2000 g 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 reagent 3 , 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.

3. Serum/plasma

(1) Prepare serum/plasma as the common method.

(2) Take 100 μL of sample and add 400 μL of reagent 3 , 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.

4. Tissue homogenate

(1) Collect fresh tissue, wash with normal saline, then absorb the water on surface of the tissue.

(2) Weigh the tissue accurately, add reagent 3 according to ratio of Weight (g): Volume (mL) =1:9 (It is recommended to take 0.05 g tissue). Homogenize mechanically with a homogenizer in ice-bath to prepare 10% homogenate.

(3) Centrifuge at 10000 g for 10 min.

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

5. Cells sample

(1) Collect the cells and wash the cells with PBS (0.01 M, pH 7.4) for 1~2 times.

(2) Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment.

(3) Add reagent 3 at a ratio of cell number (10^6): volume (μL) =1: 400 (It is recommended to take 1×10^6 cells).

(4) Sonicate or grind with hand-operated in ice water bath.

(5) Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection.

▲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 the pre-experiment and the detection range (0.12-30 μmol/L).

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 reagent 3 .

Assay protocol

▲Detailed operation steps

1. The measurement of T-GSH
   1) Blank tube: take 40 μL of reagent 3 to the 2 mL EP tube.
      Standard tube: take 40 μL of 8 μmol/L GSSG standard solution to the 2 mL EP tube.
      Sample tube: take 40 μL of pretreated sample to the 2 mL EP tube.
   2) Add 600 μL of reactive working solution to each tube and incubate at room temperature or 25℃ for 5 min.
   3) Add 200 μL of reagent 9 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 reagent 10 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.

2. The measurement of GSSG
   1) Pretreatment of solution for blank tube
      Add 20 μL of reagent 7 working solution to 100 μL of reagent 3 , mix fully with a vortex mixer, then take 100 μL of liquid to 0.5 mL EP tube and add 4 μL of reagent 8 working solution, mix fully with a vortex mixer immediately, react at 25℃ for an hour.
   2) Pretreatment of standard:
      Add 20 μL of reagent 7 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 reagent 8 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 reagent 7 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 reagent 8 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 (pretreated in Step 2.1) to the 2 mL EP tube.
   Standard tube of GSSG: take 40 μL of pretreated standard (pretreated in Step 2.2) to the 2 mL EP tube.
   Sample tube of GSSG: take 40 μL of sample supernatant (pretreated in Step 2.3) to the 2 mL EP tube.
5) Add 600 μL of reactive working solution to each tube and incubate at room temperature or 25℃ for 5 min.
6) Add 200 μL of reagent 9 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 reagent 10 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

1. For 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. For animal 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. For cultured cells samples

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

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

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 reagent 3 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 reagent 3 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 Data

▲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:

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

Appendix II References

1. Meister A, Anderson M E. Glutathione[J]. Annu Rev Biochem, 1983, 52: 711-760.

2. Corso C R, Acco A. Glutathione system in animal model of solid tumors: From regulation to therapeutic target[J]. Critical Reviews in Oncology/ Hematology, 2018, 128: 43-57.

3. Deleve L D, Kaplowitz N. Glutathione metabolism and its role in hepatotoxicity[J]. Pharmacology & Therapeutics, 1991, 52(3): 287-305.

4. Monostori P, Wittmann G, Karg E, et al. Determination of glutathione and glutathione disulfide in biological samples: An in-depth review[J]. J Chromatogr B Analyt Technol Biomed Life Sci, 2009, 877(28): 3331-3346.

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