Technical Manual

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

- Catalogue Code: MAES0086
- Size: 96T
- Research Use Only

1. Key features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

0.36-30 µmol/L T-GSH

Sensitivity:

0.36 µmol/L T-GSH

Storage:

-20°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. 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.

3. Intended Use

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

4. 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.

5. Kit components & storage

Item	Specification	Storage
Buffer Solution	45 mL × 2 vials	-20°C, 6 months
Standard	6.13 mg × 1 vial	-20°C, 6 months
Protein Precipitator	Lyophilized × 1 vial	-20°C, 6 months
Enzyme Stock Solution	80 µL × 1 vial	-20°C, 6 months
Chromogenic Agent	Lyophilized × 1 vial	-20°C, 6 months, avoid direct sunlight
Diluent	1.8 mL × 1 vial	-20°C, 6 months
GSH Scavenger Auxiliary Solution	2 mL × 1 vial	-20°C, 6 months
GSH Scavenger	0.5 mL × 1 vial	-20°C, 6 months, avoid direct sunlight
Substrate	Lyophilized × 1 vial	-20°C, 6 months, avoid direct sunlight
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (405-415 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Absolute ethanol
- PBS (0.01 M, pH 7.4)

6. Assay Notes:

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.

7. Reagent preparation:

1. Preserve enzyme stock solution on ice and other reagent to room temperature before use.

2. Preparation of **protein precipitator working solution:** Dissolve the protein precipitator (g) with buffer solution (mL) at the ratio of 1: 19 and mix fully. Prepare the fresh solution before use and the prepared solution can be stored at 2-8°C for 24 hours. (If there is an insoluble floating substance, do not affect the use.)

3. Preparation of **enzyme working solution:** (Operate on ice) Dilute the enzyme stock solution (mix fully before use) with buffer solution at the ratio of 1:19. Prepare the fresh solution before use and the prepared solution can be stored at 2-8°C for 24 hours.

4. Preparation of **chromogenic agent working solution:** Dissolve a vial of chromogenic agent lyophilized with 1.5 mL of diluent fully. Unused chromogenic agent working solution can be stored at -20°C for 3 months. It is recommended to aliquot the prepared solution into small quantities and store at -20°C.

5. Preparation of **reactive working solution:** Mix the enzyme working solution, chromogenic agent working solution and buffer solution at the ratio of 1: 1: 25. Prepare the fresh solution before use and the prepared solution can be stored at 2-8°C for 24 hours.

6. Preparation of **scavenger auxiliary working solution:** Dilute the GSH scavenger auxiliary solution 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°C for 24 hours. (Note: GSH scavenger auxiliary solution should be pipetted slowly.)

7. Preparation of **scavenger application solution:** Dilute the GSH scavenger 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°C for 24 hours.

8. Preparation of **substrate stock solution:** Dissolve a vial of substrate with 100 µL of double distilled water fully. It is recommended to aliquot the prepared solution into small quantities and store at -70°C for 3 months.

9. Preparation of **substrate working solution:** Dilute the substrate stock solution with buffer solution at the ratio of 1:79. Prepare the fresh solution before use and the prepared solution can be stored at 2-8°C for 24 hours.

10. Preparation of **standard stock solution (1 mmol/L):** Dissolve a vial of standard fully with 10 mL of double distilled water. Aliquot the stock solution into small quantities and it can be store at -20°C for 1 month.

11. Preparation of **standard solution (20 µmol/L):** Dilute standard stock solution (1 mmol/L) with protein precipitator working solution at the ratio of 1:49. Prepare the fresh solution before use and the prepared solution can be stored at 2-8°C for 24 hours.

8. Sample Preparation

Sample requirements

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

1. Serum/plasma sample:

Prepare serum/plasma as the common method. Take 100 µL of sample and add 400 µL of protein precipitator working solution, mix fully by a vortex mixer for 30 s, stand for 5 min at 4°C. Centrifuge at 3100 g for 10 min. Take the supernatant and preserve it on ice for detection.

2. Cell sample:

Collect the cells and wash the cells with PBS (0.01 M, pH 7.4) for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add protein precipitator working solution at a ratio of cell number (10^6): volume (µL) =1: 400 (It is recommended to take 1×10^6 cells). Sonicate or grind with hand-operated in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection.

3. Tissue sample:

Collect fresh tissue, wash with normal saline, then absorb the water on surface of the tissue. Weigh the tissue accurately, add protein precipitator working solution according to ratio of Weight (g): Volume (mL) =1:4 (It is recommended to take 0.05 g tissue). Homogenize mechanically with a homogenizer in ice-bath to prepare 20% homogenate. Centrifuge at 10000 g for 10 min. Take the supernatant and preserve it on ice for detection.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment

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

Sample Type:	Dilution Factor
Human plasma	1
10% Rat liver tissue homogenate	10-20
10% Rat kidney tissue homogenate	1
10% Rat heart tissue homogenate	10-20
10% Mouse brain tissue homogenate	2-5
HepG2 cells	1

Note: The diluent is protein precipitator working solution.

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 412 nm

Plate Set Up:

The measurement for T-GSH or or GSSG

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A-H, standard wells; S1-S80, sample wells.

The measurement for T-GSH or or GSSG

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	A'	A'	S1'	S9'	S17'	S25'
B	B	B	S2	S10	S18	S26	B'	B'	S2'	S10'	S18'	S26'
C	C	C	S3	S11	S19	S27	C'	C'	S3'	S11'	S19'	S27'
D	D	D	S4	S12	S20	S28	D'	D'	S4'	S12'	S20'	S28'
E	E	E	S5	S13	S21	S29	E'	E'	S5'	S13'	S21'	S29'
F	F	F	S6	S14	S22	S30	F'	F'	S6'	S14'	S22'	S30'
G	G	G	S7	S15	S23	S31	G'	G'	S7'	S15'	S23'	S31'
H	H	H	S8	S16	S24	S32	H'	H'	S8'	S16'	S24'	S32'

Note: A-H, standard wells of T-GSH; S1-S32, sample wells of T-GSH; A'-H', standard wells of GSSG; S1'-S32', sample wells of GSSG.

10. Operation Steps

The preparation of standard curve

Dilute standard solution (20 μmol/L) with protein precipitator working solution to a serial concentration. The recommended dilution gradient is as follows: 0, 0.5, 1, 2, 5, 8, 10, 15 μmol/L.

The measurement of T-GSH

1. **Standard wells:** take 10 μL of the standard solution with different concentration to the corresponding wells.
   Sample wells: take 10 μL of sample to the corresponding sample wells.

2. Add 150 μL of reactive working solution to each well and incubate at room temperature or 25°C for 5 min.

3. Add 50 μL of substrate working solution to each well, mix fully for 5 s with microplate reader.

4. Incubate at room temperature or 25°C for 25 min and measure the OD value of each well at 412 nm.

The measurement of GSSG

1. **The pretreatment of standard:** Add 20 μL of scavenger auxiliary working solution to 100 μL of the standard solution with different concentration (15, 10, 8, 5, 2, 1, 0.5, 0 μmol/L), mix fully with a vortex mixer, then take 100 μL of liquid to 0.5 mL EP tube and add 4 μL of scavenger application solution, mix fully with a vortex mixer immediately, react at 25°C for an hour.

2. **Remove the GSH of samples:** Add 20 μL of scavenger auxiliary working solution to 100 μL of samples (pretreated with protein precipitator working solution 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 scavenger application solution, mix fully with a vortex mixer immediately, react at 25°C for an hour.

3. **Standard wells of GSSG:** take 10 μL of the standard solution with different concentration (pretreated in Step 1) to the corresponding wells.
   Sample wells of GSSG: take 10 μL of sample (pretreated in Step 2) to the corresponding sample wells

4. Add 150 μL of reactive working solution to each well and incubate at room temperature or 25°C for 5 min.

5. Add 50 μL of substrate working solution to each well, mix fully for 5 s with microplate reader.

6. Incubate at room temperature or 25°C for 25 min and measure the OD value of each well at 412 nm.

11. Calculations

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample.

The standard curve of T-GSH is: $y= a_1x + b_1$.

The standard curve of GSSG is: $y= a_2x + b_2$.

1. Serum (plasma) sample:

$$\text{T-GSH content (μmol/L)} = (\Delta A_1 - b_1) \div a_1 \times 2^* \times 5^{**} \times f$$

$$\text{GSSG content (μmol/L)} = (\Delta A_2 - b_2) \div a_2 \times 5^{**} \times f$$

2. Tissue sample:

$$\text{T-GSH content (μmol/g)} = (\Delta A_1 - b_1) \div a_1 \times 2^* \div \frac{m}{V_1} \times f$$

$$\text{GSSG content (μmol/g)} = (\Delta A_2 - b_2) \div a_2 \div \frac{m}{V_1} \times f$$

3. Cell sample:

$$\text{T-GSH content } (\mu mol/10^6) = (\Delta A_1 - b_1) \div a_1 \times 2^* \div \frac{1^{***}}{V_2} \times f$$

$$\text{GSSG content } (\mu mol/10^6) = (\Delta A_2 - b_2) \div a_2 \div \frac{1^{***}}{V_2} \times f$$

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

y: $OD_{Standard} - OD_{Blank}$ (OD_{Blank} is the OD value when the standard concentration is 0).
x: The concentration of standard.
a_1: The slope of standard curve of T-GSH.
b_1: The intercept of standard curve of T-GSH.
ΔA_1: $OD_{Sample.} - OD_{Blank}$ (for T-GSH)
a_2: The slope of standard curve of GSSG.
b_2: The intercept of standard curve of GSSG
ΔA_2: $OD_{Sample.} - OD_{Blank}$ (for GSSG)
2*: With GSSG as the standard, need to multiply by 2 when converting to GSH.
5:** Dilution multiple of sample in sample preparation step.
f: Dilution factor of sample before test.
m: the fresh weight of sample.
V_1: the volume of protein precipitator working solution in sample preparation step of tissue sample.
1*:** the cell number, 1×10^6.
V_2: the volume of protein precipitator working solution in sample preparation step of cell sample.

12. Performance Characteristics

Detection Range	0.36-30 μmol/L T-GSH
Sensitivity	0.36 μmol/L T-GSH
Average recovery rate (%)	97
Average inter-assay CV (%)	3.9
Average intra-assay CV (%)	0.6

Analysis

Dilute 10% rat liver tissue homogenate with protein precipitator working solution for 20 times, then take 10 μL of diluted sample, carry the assay according to the operation table.

The standard curve of T-GSH: y = 0.0858 x + 0.0064, the average OD value of the sample well is 1.159, the average OD value of the blank well is 0.114, the calculation result is:

$$\text{T-GSH (μmol/g)} = (1.159 - 0.114 - 0.0064) \div 0.0858 \times 2 \div 0.05 \times 0.45 \times 10^{-3} \times 20$$
$$= 4.36 \text{ μmol/g}$$

The standard curve of GSSG: y = 0.0717 x – 0.007, the average OD value of the sample well is 0.320, the average OD value of the blank well is 0.118, the calculation result is:

$$\text{GSSG (μmol/g)} = (0.320 - 0.118 + 0.007) \div 0.0717 \div 0.05 \times 0.45 \times 10^{-3} \times 20$$
$$= 0.52 \text{ μmol/g}$$

Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching 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.

Before the experiment, read the instructions carefully, and wear gloves and work clothes.

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