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

Catalog No: E-BC-K097-M

Method: Colorimetric method

Specification: 96T (Can detect 80 samples for T-GSH or GSSG without duplication, or detect 32 samples for both T-GSH and GSSG without duplication)

Measuring instrument: Microplate reader

Sensitivity: 0.36 μmol/L T-GSH

Detection range: 0.36-30 μmol/L T-GSH

- Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

This kit can be used to measure T-GSH and 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	45 mL × 2 vials	-20℃ , 6 months
Reagent 2	Standard	6.13 mg × 1 vial	-20℃ , 6 months
Reagent 3	Protein Precipitator	Powder × 1 vial	-20℃ , 6 months
Reagent 4	Enzyme Stock Solution	80 μL × 1 vial	-20℃ , 6 months
Reagent 5	Chromogenic Agent	Powder × 1 vial	-20℃ , 6 months, shading light
Reagent 6	Diluent	1.8 mL × 1 vial	-20℃ , 6 months
Reagent 7	GSH Scavenger Auxiliary Solution	2 mL × 1 vial	-20℃ , 6 months
Reagent 8	GSH Scavenger	0.5 mL × 1 vial	-20℃ , 6 months, shading light
Reagent 9	Substrate	Powder × 1 vial	-20℃ , 6 months, shading light
	Microplate	96 wells	
	Plate Sealer	2 pieces	

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

Microplate reader (405-415 nm), Micropipettor, Centrifuge, Incubator, Vortex mixer

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (0.5 mL, 2 mL, 5 mL, 10 mL)

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 3 working solution

   Dissolve the powder with reagent 1 at the ratio of reagent 3 (g): reagent 1 (mL) =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.)

2. Preparation of reagent 4 working solution

   (Operate on ice) Dilute the reagent 4 (mix fully before use) 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°C for 24 hours.

3. Preparation of reagent 5 working solution

   Dissolve a vial of powder with 1.5 mL of reagent 6 fully. Unused reagent 5 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 .

4. 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°C for 24 hours.

5. 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°C for 24 hours. (Note: reagent 7 should be pipetted slowly.)

6. 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°C for 24 hours.

7. Preparation of reagent 9 stock solution

Dissolve a vial of reagent 9 with 100 μL of double distilled water fully. It is recommended to aliquot the prepared solution into small quantities and store at -70℃ for 3 months.

8. Preparation of reagent 9 working solution

Dilute the reagent 9 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.

9. Preparation of 1 mmol/L standard stock solution

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

10. Preparation of 20 μmol/L Standard solution

Dilute 1 mmol/L standard stock solution with reagent 3 working solution at the ratio of 1:49. 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 working solution, 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 working solution, 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 working solution, 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 working solution 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 working solution 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 (0.36-30 μmol/L T-GSH).

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 reagent 3 working solution.

Assay protocol	
Ambient temperature	25-30°C
Optimum detection wavelength	412 nm

Instructions for the use of transferpettor

(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.

(2) Don't add the liquid outside the tips into the reaction system when pipetting each reagent.

Assay protocol

▲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 both T-GSH and 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.

▲Operating steps

1.The preparation of standard curve

Dilute 20 μmol/L standard solution with reagent 3 working solution to a serial concentration. The recommended dilution gradient is as follows: 0, 0.5, 1, 2, 5, 8, 10, 15 μmol/L.

2. 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 reagent 9 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.

3. The measurement of GSSG

1) The pretreatment of standard:
Add 20 μL of reagent 7 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 reagent 8 working 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 reagent 7 working solution to 100 μL of samples (pretreated with reagent 3 working solution in sample preparation step), mix fully with a vortex mixer, then take 100 μL od 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°C for an hour.

3) Standard wells of GSSG: take 10 μL of the standard solution with different concentration (pretreated in Step 3.1) to the corresponding wells.
Sample wells of GSSG: take 10 μL of sample (pretreated in Step 3.2) 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 reagent 9 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.

▲Calculation

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 is: y= ax + b.

1.For serum (plasma), whole blood, red blood cells samples

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

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

2.For animal 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.For cells samples

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

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

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

Note:

y: $OD_{Standard} - OD_{Blank}$.

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.

ΔA_{412}: Absolute OD ($OD_{Sample} - OD_{Blank}$).

m: the fresh weight of sample.

V_1: the volume of reagent 3 working solution in sample preparation step of tissue sample.

1***: the cell number, 1×10^6.

V_2: the volume of reagent 3 working solution in sample preparation step of cell sample.

▲Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 6 months.
4. Do not use components from different batches of kit.

Appendix I Performance characteristics

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

▲Inter-assay CV

Take three kits of different batches to detect the samples of high concentration, middle concentration and low concentration respectively. The concentrations should be within the detection range. The samples of each concentration were tested 3 times (n=3) parallelly with each kit. Then calculate the corresponding values according to the following formula. The average Inter-assay CV is 3.9%.

$$\overline{x}_T = \frac{\overline{x}_1 + \overline{x}_2 + \overline{x}_3}{3}$$

$$R = \frac{\overline{x}_{max} - \overline{x}_{min}}{\overline{x}_T} \times 100\%$$

$$\overline{R} = \frac{R_1 + R_2 + R_3}{3} \quad (n=3)$$

$\overline{x}_{max}$--- The max values of $\overline{x}_i$

$\overline{x}_{min}$--- The min values of $\overline{x}_i$

$\overline{x}_T$---The average values of $\overline{x}_i$

R_i---The value of each batch number kit

▲Intra-assay CV

Take one kit to detect the samples of high concentration, middle concentration and low concentration respectively. The concentrations should be within the detection range. The samples of each concentration were tested 6 times (n=6) parallelly, and the average Intra-assay CV is 0.6%, which was calculated according to the following formula.

$$CV = \frac{S}{\bar{x}} \times 100\%$$

S--- Standard deviation

▲Sensitivity

The volume of sample added to reaction is 10 μL, carry the assay according to the operation table, the minimum detected absorbance is 0.005 (ΔA_{412}=0.005), calculate the sensitivity according to the formula in calculation is 0.36 μmol/L T-GSH.

▲Recovery rate

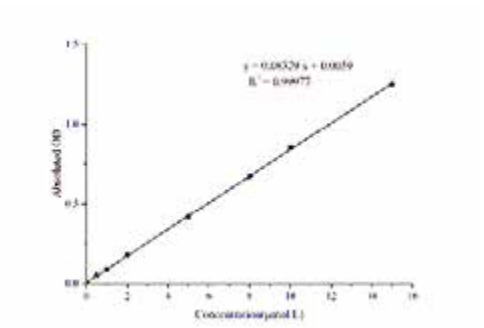
Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 3 times parallelly to get the average recovery rate of 97%.

▲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.

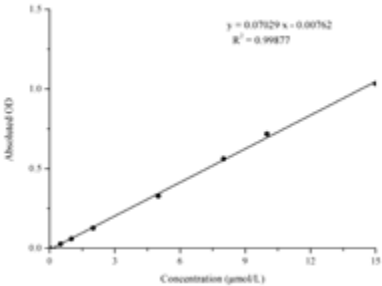
The standard curve of T-GSH is as follows:

Concentration (μmol/L)	0	0.5	1	2	5	8	10	15
Average OD	0.113	0.140	0.173	0.240	0.442	0.675	0.831	1.147
Absoluted OD	0	0.027	0.060	0.127	0.329	0.562	0.718	1.034



The standard curve of GSSG is as follows:

Concentration (μmol/L)	0	0.5	1	2	5	8	10	15
Average OD	0.105	0.156	0.194	0.281	0.524	0.773	0.957	1.355
Absoluted OD	0	0.051	0.089	0.176	0.419	0.668	0.852	1.250



▲Example analysis

Dilute 10% rat liver tissue homogenate with reagent 3 working solution for 20 times, then take 10 μL of diluted sample and carry the assay according to the operation steps. The results are as follows:

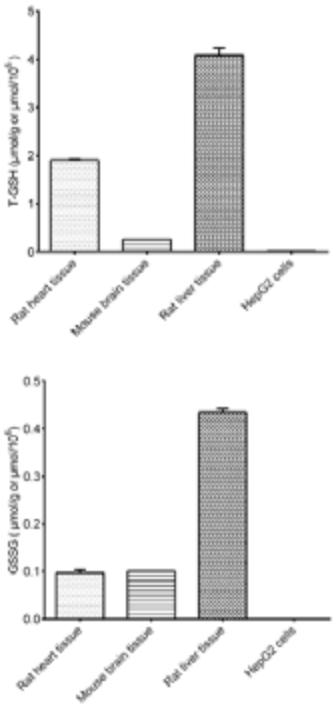
The standard curve of T-GSH: y = 0.0858 x + 0.0064, the average OD value of the sample well is 1.159, the average OD value of the blank well is 0.114, the calculation result is:

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

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:

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

Detect 10% rat heart tissue homogenate (dilute for 10 times), 10% mouse brain tissue homogenate (dilute for 2 times), 10% rat liver tissue homogenate (dilute for 20 times), HepG2 cells (dilute for 2 times) according to the protocol, the result is as follows:



Appendix II References

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