Technical Manual

Reduced Glutathione (GSH) Colorimetric Assay Kit

- Catalogue Code: MAES0042
- Size: 96T
- Research Use Only

1. Key features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

2-100 μmol/L

Sensitivity:

2 μmol/L

Storage:

2-8°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

Reduced glutathione (GSH) is a kind of low molecular scavenger, which can remove O2-, H2O2, LOOH. GSH is a small molecule peptide which composed of glutamic acid, glycine and cysteine, and it is the main thiol compound of non-protein in the organization. GSH is the substrate of GSH-Px and GSH-ST which is indispensable for decomposing hydrogen peroxide of these two enzymes, and it can stabilize the enzyme containing thiol and prevent hemoglobin and other auxiliary factors from the oxidative damage. Recently, it is proved that GSH is also involved in the recovery of VE to the reduction state. When lacking or depletion of GSH, it may cause producing toxic effects or increasing the toxic effects of many chemicals or environmental factors. It may be related to the increase of oxidative damage, so the amount of GSH is a vital factor to measure the body's antioxidant ability. GSH plays an important role in the researches process of prevention, recovery and treatment of atherosclerosis, coronary heart disease, anti-aging, anti-tumor, prevention, prevention of Alzheimer's disease and other diseases.

3. Intended Use

This kit can measure GSH content in serum, plasma, cells, cell culture supernatant and tissue samples.

4. Detection Principle

Reduced glutathione (GSH) can react with dinitrobenzoic acid (DNTB) to form a yellow complex which can be detected by colorimetric assay at 405 nm and calculate the reduced GSH content indirectly.

5. Kit components & storage

Item	Specification	Storage
Acid Reagent	12 mL × 1 vial	2-8°C,6 months, avoid direct sunlight
Phosphate	12 mL × 1 vial	2-8°C,6 months
DTNB Solution	1.5 mL × 2 vials	2-8°C,6 months, avoid direct sunlight
GSH Standard	3.07 mg × 2 vials	2-8°C,6 months
GSH Standard Stock Diluent	1.5 mL× 2 vials	2-8°C,6 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (405-414 nm)
- Tips (10 µL, 200 µL, 1000 µL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Normal Saline (0.9% NaCl)
- PBS (0.01 M, pH 7.4)

6. Assay Notes:

1. The supernatant after centrifugation must be clarified.

2. Prevent the formulation of bubbles when the supernatant is transferred into the microplate.

7. Reagent preparation:

1. Bring all reagents to room temperature before use.

2. Preparation of GSH standard diluent: Dilute the GSH standard stock diluent with double-distilled water at a ratio of 1:9. Prepare the fresh solution before use.

3. Preparation of GSH standard solution (1 mmol/L): Dissolve 3.07 mg of GSH standard with 10 mL of GSH standard diluent and mix fully. Prepare the fresh solution before use.

8. Sample Preparation

1. Serum sample:
Fresh blood should be incubated at 25°C for 30 min to clot the blood. Centrifuge the sample at 2000 g for 15 min at 4°C. Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection. If not detected on the same day, the serum can be stored at -80°C for a month.

2. Plasma sample:
Place the fresh blood sample into a tube of anticoagulant and centrifuge at 700-1000g for 10 min at 4°C. Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice for detection. If not detected on the same day, the plasma can be stored at -80°C for a month.

3. 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 homogenization medium at a ratio of cell number (2×10^6): 50 mM Tris-HCl (pH 7.4) including 150 mM NaCl, 1% NP-40, 1 mM EDTA (μL) =1: 300. Sonicate the sample on an ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. If not detected on the same day, the cells sample (without homogenization) can be stored at -80°C for a month.

4. Tissue sample:

Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C. Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of 50 mM Tris-HCl (pH 7.4) including 150 mM NaCl, 1% NP-40, 1 mM EDTA (2-8°C) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4°C. Take the supernatant to preserve it on ice for detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

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 and the detection range (2-100 μmol/L).

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

Sample Type:	Dilution Factor
Human serum	1
Human plasma	1
10% Mouse brain tissue homogenization	1
10% Mouse liver tissue homogenization	1
Hela cell homogenization（0.999 mgprot/mL）	1
Rat serum	1
Rat plasma	1
Mouse serum	1
10% Carrot tissue homogenization	1
293T supernatant	1

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4);

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 405 nm

Plate Set Up:

	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.

10. Operation Steps

The preparation of standard curve

Dilute GSH standard solution (1 mmol/L) with GSH standard diluent to a serial concentration. The recommended dilution gradient is as follows: 0, 10, 20, 40, 50, 60, 80, 100 μmol/L.

The measurement of samples

1. **Preparation of sample supernatant:** take 0.1 mL of sample, add 0.1 mL of acid reagent and mix fully. Centrifuge at 4500 g for 10 min. Take the supernatant for detection.

2. Add 25 μL DTNB solution to each well.

3. **Control well:** Add 100 μL of acid reagent.
   Standard well: Add 100 μL of standard solution with different concentration.
   Sample well: Add 100 μL of supernatant

4. Add 100 μL of phosphate to each tube.

5. Mix fully for 1 min and stand for 5 min at room temperature. Measure the OD values of each well at 405 nm with microplate reader.

Operation Table

	Standard well	Sample well	Control well
DTNB solution (μL)	25	25	25
Acid reagent (μL)			100
GSH standard solution with different concentration (μL)	100		
Supernatant (μL)		100	
Phosphate (μL)	100	100	100
Mix fully for 1 min and stand for 5 min at room temperature. Measure the OD values of each well at 405 nm with microplate reader.			

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 is: y= ax + b.

1. Serum (plasma), culture supernatant and other liquid sample:

$$\text{GSH content (μmol/L)} = (\Delta A_{405}\text{-b}) \div a \times 2^* \times f$$

2. Tissue and cells sample:

$$\text{GSH content (μmol/gprot)} = (\Delta A_{405}\text{-b}) \div a \times 2^* \times f \div C_{pr}$$

y: $OD_{Standard} - OD_{Blank}$ (OD_{Blank} is the OD value when the standard concentration is 0).
x: The concentration of standard.
a: The slope of standard curve .
b: The intercept of standard curve.
ΔA_{405}: $OD_{Sample} - OD_{Contorl}$
f: Dilution factor of sample before test.
2*: Dilution factor of in the preparation step of sample supernatant, 2 times.
C_{pr}: Concentration of protein in sample, gprot/L.

12. Performance Characteristics

Detection Range	2-100 μmol/L
Sensitivity	2 μmol/L
Average recovery rate (%)	96
Average inter-assay CV (%)	3.2
Average intra-assay CV (%)	1.9

Analysis

Take 0.1 mL of human serum sample, add 0.1 mL of acid reagent, mix fully and centrifuge at 3100 g for 10 min, then take prepared supernatant, carry the assay according to the operation table.

The results are as follows:

standard curve: y = 0.00383x – 0.00251, the average OD value of the sample is 0.080, the average OD value of the control is 0.047, and the calculation result is:

$$\text{GSH content (μmol/L)} = (0.080 - 0.047 + 0.00251) \div 0.00383 \times 2$$
$$= 18.54\ \mu\text{mol/L}$$

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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Notes:

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