Total Sulfhydryl Group/Total Thiol (-SH) Colorimetric Assay Kit

Catalog No: E-BC-K265-M

Method: Colorimetric method

Specification: 96T (Can detect 40 samples without duplication)

Measuring instrument: Microplate reader

Sensitivity: 9.91 μmol/L

Detection range: 9.91-1000 μmol/L

- 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 measure total (-SH) content in serum, plasma, animal tissue samples.

▲Background

Total sulfhydryl group/total thiol (-SH) is an important protein structure and REDOX reactive group in organisms. Sulfhydryl is one of the most active and ubiquitous ligands in biological systems. It is found in most proteins, but also in some low molecular weight substances. Sulfhydryl groups play an important role in biochemical processes, not only for the REDOX mechanism, but also for the enhancement of the function of some hydrolyzed biocatalysts. Compounds containing sulfhydryl groups are called thiols. Decreased thiol levels are found in various diseases.

▲Detection principle

Sulfhydryl compounds react with 5,5' -dithiobis (2-nitrobenzoic acid) under neutral or alkaline conditions to produce a yellow product which have a maximum absorption peak at 412 nm. Measure the OD value and calculate the total mercapto content indirectly.

▲Kit components & Storage

Item	Component	Specification	Storage
Reagent 1	Buffer Solution	20 mL× 1 vial	2-8℃ , 6 months
Reagent 2	Chromogenic Agent	1.3 mL× 1 vial	2-8℃ , 6 months, shading light
Reagent 3	Standard Powder	Powder × 2 vials	2-8℃ , 6 months
	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 (410-420 nm), Micropipettor, Water bath, Incubator, Vortex mixer, Centrifuge

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL)

Reagents:

Double distilled water, Absolute ethyl alcohol (AR)

▲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 kit contain the pungent odour reagent. Please carry the assay in a fume hood.
2. In the step of pretreatment of sample supernatant, the supernatant after centrifugation should be clarified.
3. When the protein concentration of the sample is too high, the reaction system will become turbid after the addition of the chromogenic agent. So the sample should be diluted and tested again.

Pre-assay preparation

▲Reagent preparation

Preparation of 5 mmol/L standard solution:
Dissolve a vial of reagent 3 with 10 mL of normal saline and mix fully. The prepared solution can be stored at 2-8℃ for a day.

▲Sample preparation

1. Serum sample
   Fresh blood was collected and placed at 25℃ for 30 min to clot the blood. Centrifuge the sample at 4℃ for 15 min at 2000 g, the upper yellowish clear liquid was taken as serum. Place the serum on ice for detection.
2. Plasma sample
   The fresh blood was added into the test tube containing anticoagulant (Heparin is recommended) and mixed upside down. Centrifuge the sample at 4℃ for 10 min at 700~1000 g, the upper yellowish transparent liquid was taken as the plasma, and the middle white interference layer (white blood cells and platelets) could not be absorbed. Place the plasma on ice for detection.
3. 10% tissue homogenate sample
   Weigh the tissue accurately and add normal saline at a ratio of weight (g): volume (mL) =1: 9, homogenize the tissue in ice bath, centrifuge at 10000 g for 10 min at 4℃ , then take the supernatant for measurement.

▲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 (9.91-1000 μmol/L).

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

Sample type	Dilution factor
Human plasma	4-6
Human serum	4-6
Human urine	1
10% Rat liver tissue homogenate	4-6
Rabbit serum	3-5
Porcine serum	4-6
10% Rat kidney tissue homogenate	4-6
10% Rat spleen tissue homogenate	4-6

[Note]：The diluent is normal saline (0.9% NaCl).

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

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

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S1'	S9	S9'	S17	S17'	S25	S25'	S33	S33'
B	B	B	S2	S2'	S10	S10'	S18	S18'	S26	S26'	S34	S34'
C	C	C	S3	S3'	S11	S11'	S19	S19'	S27	S27'	S35	S35'
D	D	D	S4	S4'	S12	S12'	S20	S20'	S28	S28'	S36	S36'
E	E	E	S5	S5'	S13	S13'	S21	S21'	S29	S29'	S37	S37'
F	F	F	S6	S6'	S14	S14'	S22	S22'	S30	S30'	S38	S38'
G	G	G	S7	S7'	S15	S15'	S23	S23'	S31	S31'	S39	S39'
H	H	H	S8	S8'	S16	S16'	S24	S24'	S32	S32'	S40	S40'

[Note]：A-H, standard wells; S1-S40, sample wells; S1'-S40', control wells.

▲Operating steps

1. The preparation of standard curve

Dilute 5 mmol/L standard solution with normal saline to a serial concentration. The recommended dilution gradient is as follows: 0, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8, 1 mmol/L.

2. The measurement of samples

1) Standard well: add 40 μL of standard solution with different concentrations to the corresponding wells.
Sample well: add 40 μL of sample to the corresponding wells.
Control well: add 40 μL of sample to the corresponding wells.

2) Add 150 μL of reagent 1 to each well.

3) Add 10 μL of reagent 2 to standard wells and sample wells.

4) Add 10 μL of absolute ethyl alcohol (self-prepared) to control wells.

5) Mix fully and stand for 10 min at room temperature. Then measure the OD value of each well at 412 nm with microplate reader.

▲Operation table

	Standard	Sample	Control
Standard solution with different concentrations (μL)	40		
Sample (μL)		40	40
Reagent 1 (μL)	150	150	150
Reagent 2 (μL)	10	10	
Absolute ethyl alcohol (self-prepared) (μL)			10
Mix fully and stand for 10 min at room temperature. Then measure the OD value of each well at 412 nm with microplate reader.			

▲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.Serum (plasma) sample

$$\text{Total (-SH) content (μmol/L)} = (\Delta A_{412} - b) \div a \times 1000^* \times f$$

2. Tissue sample

$$\text{Total (-SH) content (μmol/g fresh weight)} = (\Delta A_{412} - b) \div a \div (m \div V) \times f$$

Note:

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.

f: Dilution factor of sample before tested.

ΔA_{412}: $OD_{Sample} - OD_{Control}$.

1000*: 1 mmol/L=1000 μmol/L.

m: The fresh weight of sample, g.

V: The volume of normal saline in preparation step of tissue sample, mL.

▲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	9.91-1000 μmol/L	Average inter-assay CV (%)	2.5
Sensitivity	9.91 μmol/L	Average inter-assay CV (%)	2.9
Average recovery rate (%)	104		

▲Example analysis

For human serum, take 40 μL of human serum sample, dilute with normal saline for 5 times and carry the assay according to the operation table. The results are as follows:

standard curve: y = 1.5351 x - 0.0023, the average OD value of the sample is 0.216, the average OD value of the control is 0.115, and the calculation result is:

Total (-SH) content (μmol/L) = (0.216 – 0.115 + 0.0023) ÷ 1.5351 ×1000 × 5
= 336.46 μmol/L