（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K265-M

Specification：96T(40 samples)/500Assays(242 samples)

Measuring instrument：Microplate reader（410-420 nm）

Detection range：9.91-1000 μmol/L

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

This manual must be read attentively and completely before using this product.

If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623

Tell: 1-832-243-6086

Fax: 1-832-243-6017

Email: techsupport@elabscience.com

Website: www.elabscience.com

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

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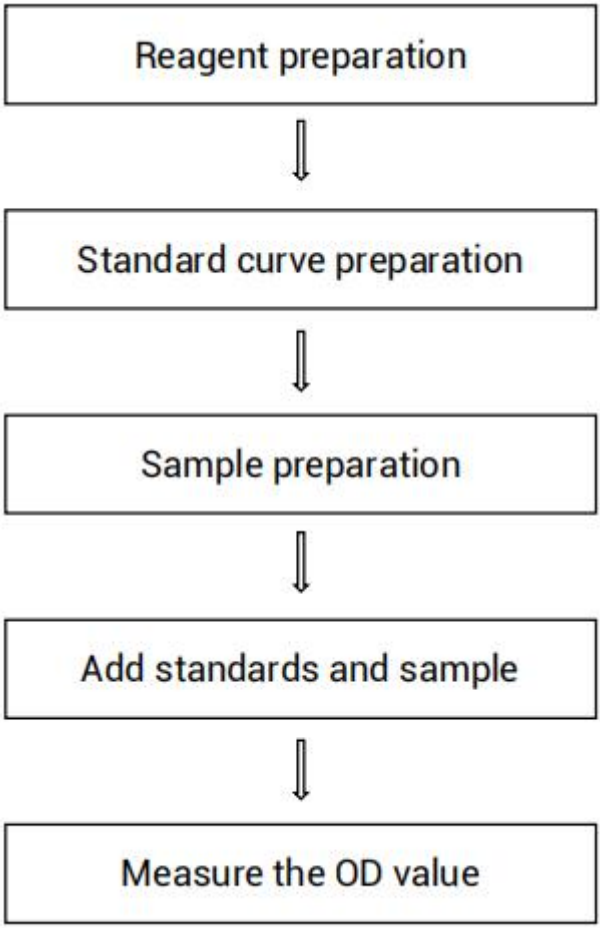
Assay summary



Intended use

This kit can be used to measure total sulfhydryl group (-SH) content in serum, plasma, animal tissue samples.

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	Size (96 T)	Size2 (500 Assays)	Storage
Reagent 1	Buffer Solution	20 mL× 1 vial	20mL×5 vials	2-8°C, 12 months
Reagent 2	Chromogenic Agent	1.3 mL× 1 vial	1.3mL×5 vials	2-8°C, 12 months shading light
Reagent 3	Standard Powder	Powder × 2 vials	Powder×10 vials	2-8°C, 12 months
	Microplate	96 wells	/	No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (410-420 nm), Micropipettor, Water bath, Incubator, Vortex mixer, Centrifuge

Reagents:

Double distilled water, Absolute ethyl alcohol

Reagent preparation

① Equilibrate other reagents to room temperature before use.

② The preparation of 5 mmol/L standard solution：

Dilute one vial of standard powder with 10 mL of normal saline, mix well. Store at 2-8°C for 1 day.

③ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

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. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.05**	**0.1**	**0.2**	**0.4**	**0.6**	**0.8**	**1.0**
5 mmol/L standard (μL)	0	10	20	40	80	120	160	200
Normal saline (μL)	1000	990	980	960	920	880	840	800

Sample preparation

① Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80°C for a month.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold normal saline (0.9% NaCl).

③ Homogenize 20 mg tissue in 180 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4°C.

④ Centrifuge at 10000×g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

② Dilution of sample

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

Sample type	Dilution factor
Human serum	4-6
Human plasma	4-6
Human urine	1
Rabbit serum	3-5
Porcine serum	4-6
10% Rat liver tissue homogenate	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). For the dilution of other sample types, please do pretest to confirm the dilution factor

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.

Operating steps

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 buffer solution to each well.

③ Add 10 μL of chromogenic agent 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.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard # ①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. Serum (plasma) sample:

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

2. Tissue sample:

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

[Note]

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.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	25.60	254.00	603.50
%CV	2.8	2.4	2.3

Inter-assay Precision

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	25.60	254.00	603.50
%CV	3.0	2.6	3.1

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 104%.

	standard 1	standard 2	standard 3
Expected Conc. (mmol/L)	0.08	0.35	0.74
Observed Conc. (mmol/L)	0.1	0.4	0.8
recovery rate(%)	101	105	106

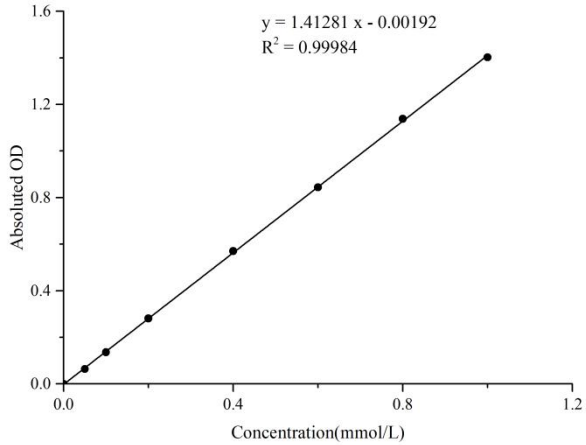
Sensitivity

The analytical sensitivity of the assay is 9.91 μmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. 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：

Concentration (mmol/L)	0	0.05	0.1	0.2	0.4	0.6	0.8	1.0
OD value	0.078	0.141	0.216	0.358	0.647	0.922	1.219	1.486
	0.077	0.142	0.213	0.358	0.649	0.921	1.214	1.474
Average OD	0.078	0.142	0.214	0.358	0.648	0.922	1.216	1.480
Absoluted OD	0.000	0.064	0.136	0.281	0.570	0.844	1.138	1.402



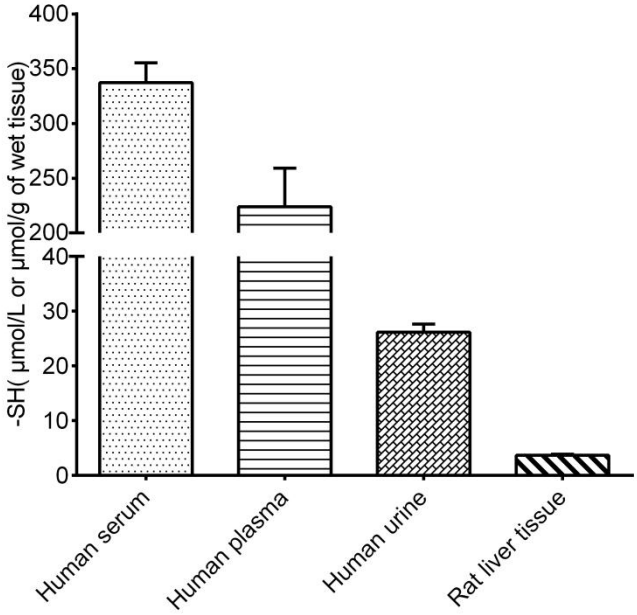
Appendix Π Example Analysis

Example analysis：

Dilute the human serum sample with normal saline for 5 times and carry the assay according to the operation steps. 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

Detect human serum (dilute for 5 times), human plasma (dilute for 5 times), human urine (dilute for 5 times) and 10% rat liver tissue homogenate (dilute for 5 times) according to the protocol, the result is as follows：



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.