

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

Catalog No: E-BC-K1403-M

Specification: 48T(32 samples)/96T(80 samples)

Measuring instrument: Microplate reader (530-550 nm)

Detection range: 0.46-100 μ mol/L

Elabscience[®] Protein Thiol 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

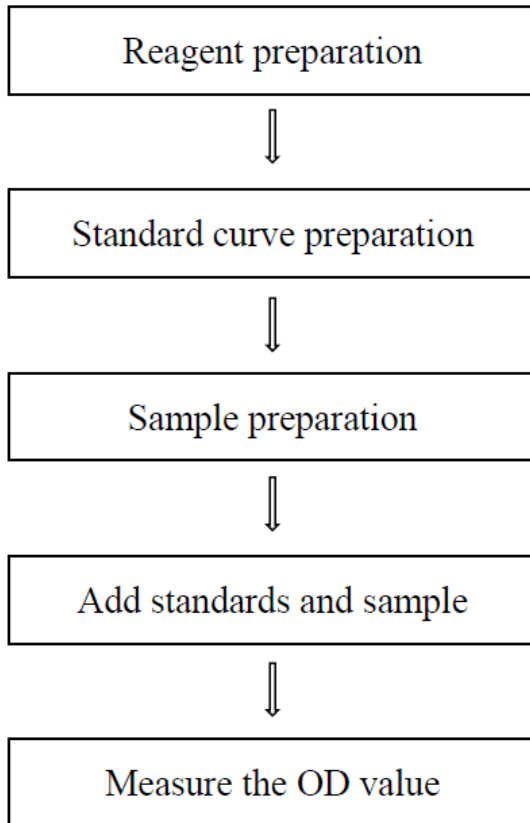
Website: www.elabscience.com

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

The kit is suitable for detecting the protein thiol in serum, plasma, urine, animal tissues and cells.

Detection principle

Protein thiol are highly abundant in living organisms and play critical roles in diverse physiological processes. Therefore, quantifying their levels in tissues and blood is of significant value for understanding the pathogenesis, treatment, prevention, and prognosis of neurodegenerative diseases.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Extraction Solution	55 mL×2 vials	55 mL×4 vials	2-8°C, 6 months, shading light
Reagent 2	Alkaline Solution	45 mL×2 vials	45 mL×4 vials	2-8°C, 6 months, shading light
Reagent 3	Buffer Solution	6 mL×1 vial	12 mL×1 vial	2-8°C, 6 months
Reagent 4	Reducing Agent	Powder×1 vial	Powder×1 vial	2-8°C, 6 months, shading light
Reagent 5	Chromogenic Agent	1.2 mL×1 vial	1.2 mL×2 vials	2-8°C, 6 months, shading light
Reagent 6	Standard	Powder×1 vial	Powder×1 vial	2-8°C, 6 months, shading light
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

Note: All the reagents should be stored according to the table. The reagents from different kits can not be mixed or used interchangeably. For liquid reagents with small volumes or powders, centrifuge them before use to prevent loss.

Instruments

Microplate reader (405-415 nm, optimum wavelength: 412 nm), Incubator (37°C)

Materials required but not provided

Double distilled water

Reagent preparation

- ① Equilibrate all the reagents to 25°C before use.
- ② Preparation of 25 mmol/L standard:
Take one vial of standard and add 1 mL of distilled double water to dissolve it completely. The 25 mmol/L standard should be stable for 5 days when stored at -20°C.
- ③ Preparation of 0.5 mmol/L standard:
Before testing, prepare a sufficient amount of 0.5 mmol/L standard according to the test wells. For example, prepare 1000 µL of a 100 µmol/L standard solution (mix 1000 µL of double distilled water and 20 µL of a 25 mmol/L standard thoroughly). The 0.5 mmol/L standard should be stable for 8 h.

④ Standard curve preparation :

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

Dilute 0.5 mmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.05, 0.1, 0.15, 0.2, 0.3, 0.4, 0.5 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	0	0.05	0.1	0.15	0.2	0.3	0.4	0.5
0.5 mmol/L Standard (μL)	0	20	40	60	80	120	160	200
Double distilled water (μL)	200	180	160	140	120	80	40	0

Sample preparation

Serum, plasma, urine and cell supernatant:

- ① Mix serum or plasma samples with Extraction Solution at a volume ratio of 1:3. Centrifuge at $850 \times g$ for 10 minutes at 4°C and discard the supernatant.
- ② Add 0.5 mL of Extraction Solution to the pellet, mix, and centrifuge again at $850 \times g$ for 5 minutes at 4°C . Discard the supernatant. Add 1.5 mL of Extraction Solution to the pellet, mix, and centrifuge at $850 \times g$ for 10 minutes at 4°C . Discard the supernatant.
- ③ Add 2 mL of Alkaline Solution to the pellet, mix, and keep on ice until analysis. The prepared samples are stable for testing within 2 days (slight turbidity is normal; mix well before assay).

Tissue sample:

- ① Homogenize 0.05 g of tissue sample in 0.45 mL of Extraction Solution. Centrifuge at $850 \times g$ for 10 minutes at 4°C and discard the supernatant.
- ② Add 0.5 mL of Extraction Solution to the pellet, mix, and centrifuge again at $850 \times g$ for 5 minutes at 4°C . Discard the supernatant. Add 1.5 mL of Extraction Solution to the pellet, mix, and centrifuge at $850 \times g$ for 10 minutes at 4°C . Discard the supernatant.
- ③ Add 2 mL of Alkaline Solution to the pellet, mix, and keep on ice until analysis. The prepared samples are stable for testing within 2 days (slight turbidity is normal; mix well before assay). Reserve an aliquot of the sample for protein concentration determination.

Cell sample

- ① Homogenize 1×10^6 cells in 500 μL of Extraction Solution. Centrifuge at $850 \times g$ for 10 minutes at 4°C and discard the supernatant.
- ② Add 0.5 mL of Extraction Solution to the pellet, mix, and centrifuge again at $850 \times g$ for 5 minutes at 4°C . Discard the supernatant. Add 1.5 mL of Extraction Solution to the pellet, mix, and centrifuge at $850 \times g$ for 10 minutes at 4°C . Discard the supernatant.
- ③ Add 2 mL of Alkaline Solution to the pellet, mix, and keep on ice until analysis. The prepared samples are stable for testing within 2 days (slight turbidity is normal; mix well before assay). Reserve an aliquot of the sample for protein concentration determination.

Dilution of sample

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

Sample type	Dilution factor
10% Mouse liver tissue homogenization	2-3
10% Mouse kidney tissue homogenization	2-3
10% Mouse heart tissue homogenization	2-3
10% Mouse spleen tissue homogenization	2-3
10% Mouse muscle tissue homogenization	2-3
Rats serum	1
Rats plasma	
Human serum	
Human plasma	1
1×10^6 RAW264.7 cells	1
1×10^6 HepG2 cells	1
1×10^6 Jurkat cells	1

Note: The diluent is Alkaline Solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

Operating steps

Incubation Steps:

① Control tube: Add 500 μL of Alkaline Solution to a corresponding 5 mL EP tube (perform only 1–2 replicates).

Test tube: Add 500 μL of the test sample to a corresponding 5 mL EP tube.

② Add 5 mg of Reducing Agent to each tube from step ① (add slowly as foam may form). Incubate at 25°C for 30 minutes with the cap open, mixing every 10 minutes.

③ Add 500 μL of Extraction Solution to each tube from step ② (add slowly as foam may form). Mix gently to obtain the test sample.

Color Development Steps:

① Standard well: Add 50 μL of each standard concentration to the corresponding wells of a microplate.

Control well: Add 50 μL of the control tube sample to the corresponding wells.

Test well: Add 50 μL of the test tube sample to the corresponding wells.

② Add 100 μL of Buffer Solution to each well from step ①.

③ Add 20 μL of Chromogenic Agent to each well from step ②

④ Shake the plate for 5 seconds, then incubate at 25°C for 5 minutes.

Measure the OD value of each well at 412 nm using a microplate reader and record as A.

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 Corrected OD value.
3. Plot the standard curve by using Corrected 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:

Serum and plasma:

$$\text{Protein Thiol content} \begin{matrix} (\mu\text{mol/L}) \end{matrix} = (\Delta A - b) \div a \times f$$

Tissue and cell sample:

$$\text{Protein Thiol content} \begin{matrix} (\mu\text{mol/kg wet weight}) \end{matrix} = (\Delta A - b) \div a \div C_{pr} \times f$$

[Note]

ΔA : Corrected OD, $OD_{\text{Sample}} - OD_{\text{Blank}}$.

C_{pr} : Protein concentration of tissue or cell sample, gprot/L.

f: Dilution factor of sample before test.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

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

Three human serum samples were assayed in replicates of 20 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.1	0.2	0.4
%CV	3.9	3.0	2.8

Inter-assay Precision

Three human urine samples were assayed 20 times in duplicate by three operators to determine precision between assays.

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 (mmol/L)	0.1	0.2	0.4
%CV	9.8	9.0	8.1

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 96%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (mmol/L)	0.1	0.2	0.4
Observed Conc. (mmol/L)	15.9	31.5	62.4
Recovery rate (%)	96	96	96

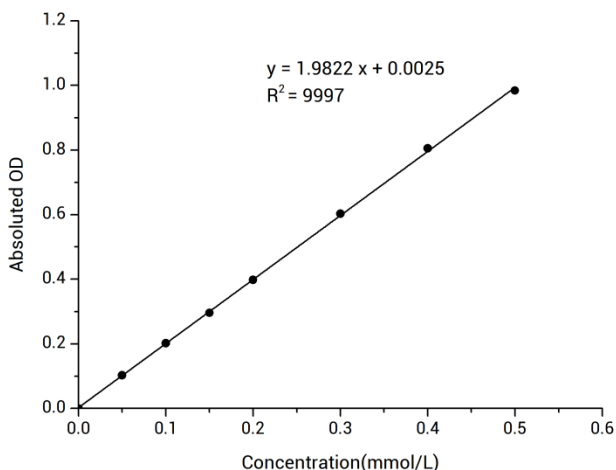
Sensitivity

The analytical sensitivity of the assay is 0.007 mmol/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 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	0.15	0.2	0.3	0.4	0.5
OD	0.105	0.208	0.306	0.405	0.504	0.705	0.913	1.082
	0.106	0.208	0.309	0.398	0.503	0.711	0.908	1.097
Average OD	0.106	0.208	0.308	0.402	0.504	0.708	0.911	1.090
Corrected OD	0	0.103	0.202	0.296	0.398	0.603	0.805	0.984



Appendix II Example Analysis

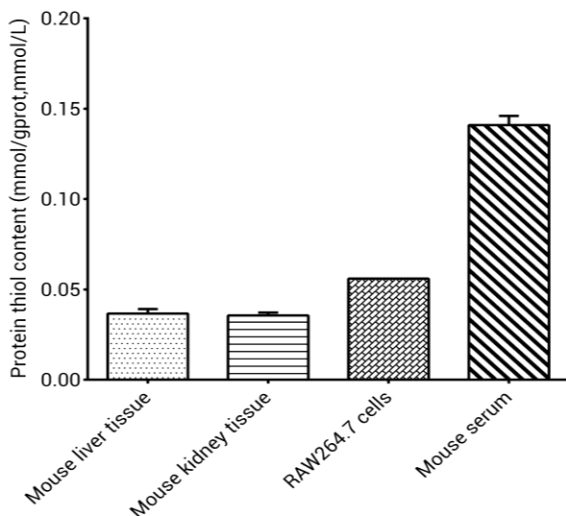
Example analysis:

Take 500 μL of the 10% mouse liver tissue sample diluted 2-fold. to the well of microplate. Proceed according to the operating steps. The results are as follows:

standard curve: $y = 1.9822x + 0.0025$. The OD of control is 0.114, the OD of sample is 0.301, $0.301 - 0.114 = 0.187$. The protein concentration of the 10% mouse liver tissue homogenate is 5.11 gprot/L and the calculation result is:

$$\text{protein thiol}(\text{mmol/gprot}) = (0.187 - 0.0025) \div 1.9822 \div 5.11 \times 2 = 0.036 \text{ mmol/gprot}$$

Detect mouse liver, mouse kidney, RAW264.7, mouse serum, , 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.

