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

Catalog No.: BC00022

Size: 100T

If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉Email (Sale) order@enkilife.com

✉Email (Techsupport) techsupport@enkilife.com

Tel: 0086-27-87002838

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Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

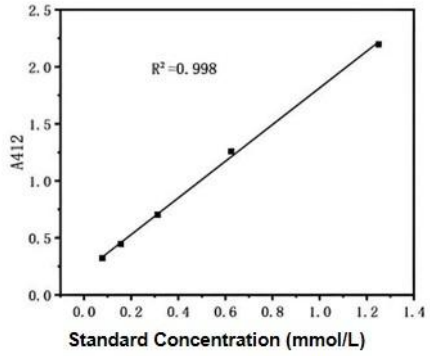
Product Name	Total Sulfhydryl Group/Total Thiol (-SH) Assay Kit
Detection Methods	Colorimetric
Sample type	Serum, plasma , tissue
Detection Type	Quantitative
Detection instrument and wavelength	Microplate reader (410-420 nm, optimal detection wavelength 412 nm)
Range	0.078125-1.25mM
Sensitivity	0.0059mM

Product Introduction

Active thiol (SH) mainly exists in cysteine, which is an important group in protein structure and certain redox reactions in organisms. The disulfide bond formed by the dehydrogenation of two thiol groups in the protein structure allows adjacent polypeptides to be connected. It is very important for maintaining the integrity of the protein structure. All animal tissues contain different amounts of active thiol groups. Glutathione, which plays an important role in the body, mainly relies on the reduction effect of the active thiol groups in the cysteine it contains. Thiol groups are also active groups of many enzymes. Some heavy metal salts such as Hg2+ bind to enzyme thiol groups and affect enzyme activity. Thiol compounds are closely related to many functional activities of the body, the effects of drugs and poisons, and the occurrence and development of certain diseases. The determination of thiol content in tissues and blood has been increasingly valued by people in order to explore the research significance in the occurrence mechanism, treatment, prevention and prognosis of certain diseases.

Detection Principle

The mercapto compounds react with 5,5'-dithiobis(2-nitrobenzoic acid) to generate a yellow product under neutral or alkaline conditions with a maximum absorption peak at 412 nm.
The figure below shows the standard curve for the determination of total thiol groups by this kit . The following standard curve is for reference only:



Product composition

Serial number	Product Name	Packing Specifications (100T)	Storage
Reagent 1	Buffer	20 mL	store at 2-8℃ after opening
Reagent 2	Color developer	1.3 mL	store at 2-8℃ after opening
Reagent 3	Total Thiol Standards	powder	store at 2-8℃ after opening
Reagent 4	Dissolving agent	20ml	store at 2-8℃ after opening
Consumables 1	96-well ELISA plate	1 plate	RT
Consumables 2	96-well membrane	2 pieces	RT

Storage conditions

The unopened kit can be stored at -20℃ for 12 months.

After opening the bottle, it can be stored at 2-8 °C and is valid for 6 months.

Preparation before the experiment

Sample processing

1. Serum and plasma samples: can be measured directly.
2. Dilution of samples : Before formal testing, 2-3 samples with large expected differences need to be selected and diluted into different concentrations for preliminary experiments .
3. Tissue sample: Take 0.020-1.0 g of fresh tissue block, add physiological saline (0.9% NaCl) at a ratio of weight (g): volume (mL) = 1:9, homogenize, centrifuge at 10000×g for 10 min at 4°C, take the supernatant and place it on ice for testing

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

· Preparation of the kit

1. Before testing, the reagents in the kit were equilibrated to room temperature.
2. 10mmol/L standard : Mix reagent 3 : reagent 4 = 0.1536g : 50ml , and let stand for 10 minutes to make the working solution. It can be stored at 2-8 ℃ for 7 days.
3. Dilution of standards of different concentrations: Mix the 10 mmol/L standard prepared in the previous step thoroughly and dilute it with double distilled water to different concentrations such as 1.0, 0.8, 0.6, 0.4, 0.2, 0 (blank well) mmol/L.

Operation Process

1. Standard wells: Take 40 μL of standard solution of different concentrations and add it to the corresponding standard wells. Assay wells: Take 40 μL of sample and add it to the corresponding assay wells.
2. 140 μL of buffer and 20 μL of colorimetric solution to the standard wells and assay wells in step "1" .
3. 412 nm using an enzyme reader .

	Standard well	Determination well
Standard solutions of different concentrations (μL)	40	--
Sample to be tested (μL)	--	40
Buffer (μL)	140	140
Color development solution (μL)	20	20
The OD value of each well was measured at 412 nm using an enzyme-labeled instrument.		

Result Calculation

Standard fitting curve: y = ax + b

Normal serum (plasma) sample, total thiol concentration calculation formula: total thiol content (mmol/L) = (ΔA412 - b) ÷ a × f

The calculation formula of total thiol (-SH) content in tissue samples is:

Total thiol content (μmol/g tissue wet weight) = (ΔA412 - b) ÷ a ÷ m/V × f

Annotation:

y: OD value of standard well - OD value of blank well (OD value when the concentration of standard

is 0)

x: concentration corresponding to the absorbance

a: slope of the curve

b: intercept of the curve

ΔA 412: Sample OD value - blank OD value (OD value when the standard concentration is 0)

f: dilution factor of the sample before adding it to the detection system

m: wet weight of tissue, it is recommended to take 0.1 g

V: The volume of homogenate medium added during tissue processing. It is recommended that V be 0.9 mL

Notes

1. The optimal detection wavelength of the microplate reader is 412 nm, and detection can be performed in the range of 410 nm- 420 nm.
2. This product is limited to scientific research by professionals and must not be used for clinical diagnosis or treatment, used as food or medicine, or stored in ordinary residences.