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

Typing thiol (-SH) Assay Kit

Catalog No.: BC00201

Size: 24T

Please read the instructions carefully before use. 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

Website: www.enkilife.com

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	Typing thiol (-SH) Assay Kit
Detection Method	Colorimetric
Sample Type	Tissue, serum, plasma, cell
Assay Type	Quantitative
Detection	Microplate reader (405 nm)

Product Introduction

Active sulfhydryl groups (SH) are mainly present in cysteine and serve as important functional groups for protein structure and certain redox reactions in organisms. In protein structures, disulfide bonds formed by dehydrogenation of two sulfhydryl groups link adjacent polypeptides, which is critical for maintaining the intact conformation of proteins.

All animal tissues contain varying amounts of active sulfhydryl groups. Glutathione, which plays a vital physiological role in the body, exerts its reducing effect mainly through the active sulfhydryl group contained in its cysteine residue. Sulfhydryl groups also act as the active functional groups of many enzymes. Heavy metal salts such as Hg^{2+} can bind to the sulfhydryl groups of enzymes and thereby inhibit enzyme activity.

Sulfhydryl compounds are closely associated with numerous physiological activities of the body, the actions of drugs and toxicants, as well as the occurrence and progression of certain diseases. The determination of sulfhydryl content in tissues and blood has attracted increasing attention, aiming to explore its research significance in the pathogenesis, treatment, prevention and prognosis evaluation of certain diseases.

Principle

5,5'-Dithiobis-(2-nitrobenzoic acid) (DTNB) reacts with sulfhydryl compounds to produce a yellow compound, which can be determined quantitatively by colorimetry.

Components

No.	Components	Size	Notes
Reagent 1	Standard powder	3 vials	Store at 4°C
Reagent 2	Clearing agent	2 mL	Seal and store at 4°C. The solution may solidify in low temperature environments. Before each assay, warm appropriately in a water bath to accelerate dissolution until the liquid becomes clear prior to use.
Reagent 3	Buffer	20 mL	Store at 4°C
Reagent 4	Chromogenic reagent	3 mL	Store at 4°C and protect from light.
Reagent 5	Precipitating reagent	10 mL	Store at room temperature. This is a supersaturated solution, if crystals precipitate, directly take the supernatant for use.
Consumable 1	Microplate	1 plate	RT
Consumable 2	Plate Sealer	2 pieces	RT

Preparation of Working solution: Mix Reagent 2 and Reagent 3 at a ratio of 1:50, and prepare only the required amount for use. Note: Calculate the dosage based on the number of samples to be tested plus the number of standard wells and blank standard wells, and prepare the working solution with an extra 2 wells allowance (to avoid insufficient reagent volume at the end of pipetting). The working solution should be freshly prepared for immediate use and can be stored for a maximum of 1-2 days.

Preparation of 10 mmol/L standard stock solution: Before each assay, dissolve one vial of standard powder with 1 mL of Reagent 3 to prepare a 10 mmol/L standard stock solution, then seal and store at 4°C.

Preparation of 500 μmol/L standard working solution: Dilute the 10 mmol/L standard stock solution with Reagent 3 at a ratio of 1:19 (20-fold dilution) to obtain the standard working solution. Prepare fresh as required immediately before use.

Storage

The kit has a validity period of 6 months.

Preparation

Microplate reader with 405 nm wavelength, 37 °C water bath or constant temperature incubator, tabletop low-speed centrifuge, pipettes of various specifications, double distilled water, normal saline (0.9%) or 0.1 M PBS, vortex mixer, test tubes or centrifuge tubes, protein assay reagents (for tissue and cell samples, available from our company).

- **Sample handling**

1. Serum (Plasma) Sample Pretreatment
   Total sulfhydryl: Directly take the sample for determination.
   Non-protein sulfhydryl:
   Test sample: Pipette 50 μL of serum (plasma), add 150 μL of Reagent V precipitant (the ratio can be adjusted appropriately at sample: Reagent V = 1:3). Mix thoroughly, centrifuge at 3500–4000 rpm for 10 min, then take 10 μL of the supernatant for testing.
   Test standard: Pipette 50 μL of 500 μmol/L standard working solution, add 150 μL of Reagent V precipitant, mix well, and take 10 μL for testing (the concentration of the standard working solution at this time is 125 μmol/L).

2. Pretreatment of Animal and Plant Tissues and Cultured Cells
   Animal Tissue: Accurately weigh the animal tissue sample. According to the mass-volume ratio (animal tissue: normal saline = 1 g: 9 mL), cut the tissue into pieces, add normal saline, and prepare a 10% tissue homogenate using a homogenizer or homogenization tube. Centrifuge at 2500–3000 rpm for 10 minutes, and collect the supernatant (10% tissue homogenate) for subsequent detection.
   Plant Tissue: Accurately weigh the plant tissue sample. According to the mass-volume ratio (plant tissue: 0.1 mol/L phosphate buffer, pH 7–7.4 = 1 g: 9 mL), cut the tissue into pieces, add 0.1 mol/L phosphate buffer (pH 7–7.4), and prepare a 10% plant tissue homogenate using a homogenizer or homogenization tube. Centrifuge at 3500–4000 rpm for 10 minutes, and collect the supernatant (10% tissue homogenate) for subsequent detection.
   Cultured Cells: Digest the cells with trypsin (or directly scrape off the cells). Transfer the cell suspension into a test tube, centrifuge at 1000–3000 rpm for 5 minutes, discard the

supernatant, and retain the cell pellet. Add 1 mL of normal saline, gently pipet to mix thoroughly to remove residual trypsin and culture medium. Centrifuge again at 1000–3000 rpm for 5 minutes, discard the supernatant, and keep the cell pellet. Add 0.2–0.3 mL of normal saline for homogenization.

Total sulfhydryl: Take the sample directly for determination.
Non-protein sulfhydryl:
Test sample: Pipette 50 μL of homogenate, add 150 μL of Reagent V precipitant (the ratio can be adjusted appropriately at sample: Reagent V = 1:3). Mix thoroughly, centrifuge at 3500–4000 rpm for 10 min, then take 10 μL of the supernatant for testing.
Test standard: Pipette 50 μL of 500 μmol/L standard working solution, add 150 μL of Reagent V precipitant, mix well, and take 10 μL for testing (the concentration of the standard working solution at this time is 125 μmol/L).

Operation process

1. Total Sulfhydryl Assay Table:

	Blank well	Standard well	Assay well
Reagent 3 (μL)	10		
500 μmol/L standard solution (μL)		10	
Sample (μL)			10
Working solution (μL)	100	100	100
Gently shake the microplate, let it stand for 5 minutes, then measure the absorbance value A1 of each well with a microplate reader at 405 nm.			
Reagent 4 (μL)	50	50	50
Gently shake the microplate and let it stand for 5 minutes. Measure the OD value A2 of each well using a microplate reader at a wavelength of 405 nm, and calculate △A = A2 − A1.			

2. Non-protein Sulfhydryl Assay Table：

	Blank well	Standard well	Assay well
Reagent 5 (μL)	10		
125 μmol/L standard solution (μL)		10	
Sample (μL)			10
Working solution (μL)	100	100	100
Gently shake the microplate, let it stand for 5 minutes, then measure the absorbance value A1 of each well with a microplate reader at 405 nm.			
Reagent 4 (μL)	50	50	50
Gently shake the microplate and let it stand for 5 minutes. Measure the OD value A2 of each well using a microplate reader at a wavelength of 405 nm, and calculate △A = A2 − A1.			

Notes:

1. For operations in a 96-well microplate, no air bubbles shall be generated in the reaction solution (air bubbles will interfere with absorbance readings). The differential pipetting method is recommended when adding working solution.
2. If the measured ΔA value is low (or close to the ΔA value of the blank well), it indicates a low sulfhydryl content in the sample. In this case, the sample loading volume (as well as the addition volume of Reagent 3/Reagent 5 in blank wells and standard solution in standard wells) can be increased (e.g., to 20 μL or 50 μL), while keeping the volumes of other reagents unchanged. Meanwhile, the standard solution concentration should be diluted by the same fold as the increase in sample loading volume. Use the diluted standard solution concentration for final calculation.

Calculation

1. Calculation for serum (plasma) samples

$$\text{Total sulfhydryl content (μmol/L)} = \frac{(\Delta A_{Assay} - \Delta A_{Blank})}{(\Delta A_{Standard} - \Delta A_{Blank})} \times C_{Standard}$$

$$\text{Non-protein sulfhydryl content (μmol/L)} = \frac{(\Delta A_{Assay} - \Delta A_{Blank})}{(\Delta A_{Standard} - \Delta A_{Blank})} \times C_{Standard} \times N$$

Protein-bound sulfhydryl (μmol/L) = Total sulfhydryl − Non-protein sulfhydryl

2. Calculation for tissue samples

$$\text{Total sulfhydryl content (μmol/L)} = \frac{(\Delta A_{Assay} - \Delta A_{Blank})}{(\Delta A_{Standard} - \Delta A_{Blank})} \times C_{Standard} \div Cpr$$

$$\text{Non-protein sulfhydryl content (μmol/L)} = \frac{(\Delta A_{Assay} - \Delta A_{Blank})}{(\Delta A_{Standard} - \Delta A_{Blank})} \times C_{Standard} \times N \div Cpr$$

Protein-bound sulfhydryl (μmol/L) = Total sulfhydryl − Non-protein sulfhydryl

Annotation:

$C_{Standard}$: Concentration of Total Sulfhydryl / Non-protein Sulfhydryl Standard, μmol/L

N: Dilution multiple of sample before determination

Cpr: Tissue homogenate protein concentration, g/L

Precautions

1. It is recommended to use disposable laboratory test tubes for operation.
2. This product is intended for scientific research use only by professionals and must not be used for clinical diagnosis or treatment, in food or drugs, or stored in ordinary residences.