Non-Protein Sulfhydryl Content Assay Kit - Microplate Method

Product Code: 112906

Product Introduction

Thiols in organisms mainly include non-protein thiols and protein thiols. Thiol compounds play important detoxification roles in vivo and are physiologically important for self-regulation.

The thiol group reacts with 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB) to form a yellow compound with a maximum absorption peak at 412 nm.

Reference test result: 2-fold diluted porcine liver sample. OD412 nm: Control 0.064; Sample 0.314/0.323. Actual readings may vary depending on the instrument and test conditions. These data are for reference only.

Package Contents and Storage

Pack size: 100T

Item Code	Component	Volume / Amount	Storage
112906.1	Reagent I	20 mL	2-8℃
112906.2	Reagent II	0.75 mL	Protect from light, 2-8℃
112906.3	Extraction Solution	110 mL	Protect from light, 2-8℃
112906.4	Standard	10 mg	Protect from light, 2-8℃
112906.m	Manual	1 copy	-

Quality Standards and Safety Instructions

Raw Material and Packaging Name	Quality Standard	Main Toxicity
Reagent I	——	——
Reagent II	——	——
Extraction Solution	——	——
Standard	——	——

Transportation and Storage Conditions

Transportation: This product is shipped with ice packs.

Storage: Store according to the instructions above.

Shelf life: 180 days.

Instructions for Use

1. Sample Preparation

1. **Tissue samples:** Use a tissue mass (g) to extraction solution volume (mL) ratio of 1:5 to 1:10. It is recommended to weigh about 0.1 g tissue and add 1 mL extraction solution. Homogenize in an ice bath, then centrifuge at 8000 g, 4℃ for 10 min. Collect the supernatant and keep it on ice for testing.

2. **Serum or culture medium:** Mix 0.5 mL sample with 0.5 mL extraction solution. Mix well and let stand at room temperature for 10 min, then centrifuge at 8000 g, 4℃ for 10 min. Collect the supernatant and keep it on ice for testing.

2. Reagent Preparation

Standard: The standard contains 10 mg reduced glutathione. Before use, add 1.3 mL extraction solution to dissolve it and prepare a 25 μmol/mL standard solution. Store at 2-8℃ for 4 weeks.

3. Procedure

1. Preheat the microplate reader for 30 min, set the wavelength to 412 nm, and zero with distilled water.
2. Dilute the 25 μmol/mL standard solution with extraction solution to prepare standard solutions of 1, 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625, and 0.0078125 μmol/mL.

Standard Solution Dilution Table

No.	Concentration Before Dilution (μmol/mL)	Standard Solution Volume (μL)	Extraction Solution Volume (μL)	Concentration After Dilution (μmol/mL)
1	25	8	192	1
2	1	100	100	0.5
3	0.5	100	100	0.25
4	0.25	100	100	0.125
5	0.125	100	100	0.0625
6	0.0625	100	100	0.03125
7	0.03125	100	100	0.015625
8	0.015625	100	100	0.0078125

Each standard tube in the following experiment requires 40 μL standard solution. Do not directly measure absorbance in this step.

4. Assay Procedure

Component	Control Tube	Test Tube	Standard Tube	Blank Tube
Sample (μL)	40	40	-	-
Standard (μL)	-	-	40	-
Distilled Water (μL)	-	-	-	40
Reagent I (μL)	150	150	150	150
Reagent II (μL)	-	10	10	-
Anhydrous Ethanol (μL)	10	-	-	10

Mix well, incubate at 25℃ for 10 min, then measure the absorbance at 412 nm. Record the values as $A_{control}$, A_{assay}, $A_{standard}$, and A_{blank}.

Calculate $\Delta A = A_{assay} - A_{control}$, and $\Delta A_{standard} = A_{standard} - A_{blank}$.

For each assay tube, set one control tube. The standard curve and blank tube only need to be measured 1-2 times.

5. Calculation of Non-Protein Sulfhydryl Content

5.1 Standard curve: Plot the concentration of the standard tube (X, μmol/mL) against absorbance (Y, $\Delta A_{standard}$) to establish the standard curve. Use the sample ΔA value (Y, ΔA) in the standard curve equation to calculate sample concentration X (μmol/mL).

5.2 Calculation formulas:

- Calculated by sample mass: Non-protein sulfhydryl content (μmol/g mass) = $X \times V_{extract} \div W \times F = X \div W \times F$
- Calculated by serum (plasma) or other liquid volume: Non-protein sulfhydryl content (μmol/mL) = $X \times (V_{liquid\ extract} + V_{liquid}) \div V_{liquid} \times F = 2 \times X \times F$
- Calculated by protein concentration: Non-protein sulfhydryl content (μmol/mg prot) = $X \times V_{extract} \div (Cpr \times V_{extract}) \times F = X \div Cpr \times F$

Symbol	Meaning
$V_{extract}$	Total volume of sample extract, 1 mL
W	Sample mass, g
Cpr	Sample protein concentration, mg/mL
$V_{liquid\ extract}$	Total volume of liquid sample extract, 0.5 mL
V_{liquid}	Serum (plasma) or other liquid volume, 0.5 mL
F	Sample dilution factor

Precautions

1. Before the formal assay, select 2-3 samples expected to show large differences for preliminary testing. This 100T kit can test 48 samples.
2. Required instruments and supplies: benchtop centrifuge, microplate reader, constant-temperature water bath, 96-well plate, adjustable pipette, mortar/homogenizer, anhydrous ethanol, ice, and distilled water.
3. The linear detection range of this kit is 0.0078125-1 μmol/mL.
4. If the measured absorbance exceeds the linear range, increase the sample volume or dilute the sample before measurement.
5. The extract contains a protein precipitant, so the supernatant cannot be used for protein concentration determination. If protein content must be measured, use a separate tissue sample.

Appendix

> The standard curve is more accurate when prepared by the user. Based on the operation table above, users may use the standard curve formula or the absorbance values of each standard well to plot a standard curve ($R^2 \geq 0.99$) and obtain the formula for sample calculation.